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# EXPERIMÉNTAL TRYPANOSOMIASIS: ITS APPLICATION IN CHEMOTHERAPEUTIC INVESTIGATIONS.

By LOUISE PEARCE, M.D., and WADE H. BROWN, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

Plates 4 to 16.

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Although experimental trypanosomiasis has been the subject of countless contributions to the literature, comparatively little has been written regarding the biological principles involved in the adaptation of the experimental infection to chemotherapeutic investigations. There have been numerous reports dealing with the more or less successful treatment of experimental trypanosomiasis beginning with Laveran and Mesnil (1), who showed in 1902 that arsenite of soda had a marked although only a temporary beneficial effect on the course of nagana infections in mice. 2 years later Ehrlich and Shiga (2) published their remarkable results with trypan red with which they were able to cure mice infected with caderas. This contribution marked the beginning of a systematic search for synthetic compounds which would exercise a specific therapeutic effect upon this class of infections, but despite the extensive investigations that have been directed toward this end during the past 15 years, the basic concepts underlying the adaptation of experimental trypanosomiasis to chemotherapeutic investigations have remained rather obscure. The experimental procedure employed by different investigators has been of the most diverse character, yet little has come out of these years of experience that would aid one either to a more judicious use of the material at his disposal or in the correct interpretation of therapeutic results obtained. The available information, such as it is, is so diffusely scattered through the literature that it is a well-nigh impossible task for one not thoroughly conversant with the facts to assemble it or to discriminate among the bewildering mass of statistics, reports, inferences, and statements with which he is at once confronted. In

view of this situation, we have attempted to present some of the salient features of experimental trypanosomiasis together with an analysis of the biological factors underlying its adaptation to chemotherapeutic investigations.

### *Trypanosomiasis in Nature.*

Trypanosomiasis comprises a group of widespread infectious conditions, some form of which is found in practically all parts of the world. Both warm and cold blooded animals are naturally susceptible to infection with one or another species of the organism. In some instances, it is an apparently harmless parasitic condition, while in others, the infection produces grave manifestations of disease. For medical and economic reasons, the greatest interest has naturally been directed toward those forms of the disease affecting man and the domestic animals. Chief among these are the human infections with *Tr. gambiense* and *Tr. rhodesiense* which cause the so called sleeping sickness of Africa, and the animal infections due to *Tr. brucei*, *Tr. evansi*, *Tr. equiperdum*, *Tr. equinum*, *Tr. dimorphon*, *Tr. theileri*, and *Tr. congolense*. Of these organisms, *Tr. brucei*, which causes the nagana of wild and domestic animals of Africa, *Tr. dimorphon*, known as the causative agent of Gambian horse disease, producing a chronic infection in other domestic animals as well, *Tr. theileri*, the causative agent of galziekte or bile fever in cattle, and *Tr. congolense*, which produces a chronic infection in horses, cattle, sheep, and dromedaries, are all confined to Africa. *Tr. evansi*, the causative agent of surra, is much more widely distributed, being found throughout Southern Asia and the adjacent islands, as well as Northern Africa. The mal de caderas in horses produced by *Tr. equinum* is confined to certain sections of South America, while dourine of horses produced by *Tr. equiperdum* is widely distributed through Africa, Asia, and certain portions of the United States, Canada, and Southern Europe.

As a class, these diseases both of man and of animals are characterized by general constitutional disturbances such as febrile periods during which the infecting organisms may be present in the blood, by anemias, emaciation, and weakness, and by general or localized edema together with a variable degree of localized inflammatory reaction

and granulomatous and ulcerative lesions. In most of these diseases, the mortality is extremely high, in some uniformly fatal, while in others, the course of the infection is relatively mild.

In general, the trypanosomes causing these diseases can be successfully inoculated into such animals as mice, rats, guinea pigs, rabbits, cats, dogs, monkeys, sheep, goats, pigs, horses, donkeys, mules, and cattle, thus affording a great variety of experimental infections capable of endless propagation. The ease with which these experimental infections may be produced is extremely variable as is the character of the resulting infections which may vary from an acutely fatal infection of a few days' duration in mice to marked chronic infection of many months' duration in the larger animals.

*Experimental Infections Employed by Other Investigators.*

During recent years, many of these experimental infections have found wide application in the chemotherapy of experimental trypanosomiasis. Experimental nagana has perhaps been most frequently employed for such purposes. The French and German investigators have used mice for most of their experiments, supplemented in many instances by larger animals, while many of the English workers have selected the rat as the animal of choice for all routine work.

Ehrlich and Shiga (2) used mice infected with nagana and caderas for the majority of their experiments. Roehl (3), working with atoxyl and arsenophenylglycine in Ehrlich's laboratory, used mice infected with the "ferrox" strain of nagana for most of his experiments, but carried out some experiments also on rabbits and guinea pigs infected with nagana and on rabbits infected with dourine. Schilling (4), also working with arsenophenylglycine, used nagana infections in mice, rats, dogs, and horses, but again the bulk of the work was done with mice. Browning (5) used mice infected with nagana and with dourine in testing arsacetin, and Morgenroth and Halberstädter (6) used nagana mice.

Laveran and Mesnil (1) in their first chemotherapeutic experiments worked with nagana mice. Nicolle and Mesnil (7) in their work with the benzidine derivatives used mice infected with *Tr. brucei*, *Tr. evansi*, and *Tr. equinum*. Laveran (8) in his experiments with arsenious acid and trypan red used mice, rats, dogs, and monkeys and in working with atoxyl, arsacetin, arsenic trisulfide, and tartar emetic, used guinea pigs infected with *Tr. dimorphon* and *Tr. congolense*. More recently, Laveran and Roudsky (9) in testing the activity of galyl used eight strains of trypanosomes in mice and but one in guinea pigs. Lafont and Dupont (10) used rats in their work with galyl and ludyl. In most of the experiments

with his new synthetic compounds 88[2] and 102[1] (luargol), Danysz (11) used mice infected with *Tr. evansi*, *Tr. rhodesiense*, *Tr. gambiense*, and *Tr. dimorphon*, although he also reports some experiments with surra rabbits. Yakimoff and Wassilevsky (12) selected dourine mice for their experiments with luargol.

The English investigators, Breinl and Nierenstein (13), recommend the rat as the animal of choice, stating that the most conclusive evidence can be obtained with this animal. Their experiments were carried out with infections produced by *Tr. brucei*, *Tr. equiperdum*, and *Tr. gambiense* in rats, guinea pigs, donkeys, and monkeys. Plimmer and Thomson (14) selected rats infected with nagana and surra for their work with antimony compounds. Wenyon (15) conducted a long series of experiments with benzidine dyes on mice infected with *Tr. dimorphon*, and Seidelin (16) tested the therapeutic activity of salvarsan-copper in rats infected with *Tr. brucei*. On the other hand, in contrast to the somewhat limited procedure of many workers, Thomas and Breinl (17) in their therapeutic experiments with atoxyl and trypan red employed a considerable number of trypanosomal infections (*Tr. gambiense*, *Tr. evansi*, *Tr. brucei*, *Tr. equinum*, *Tr. equiperdum*, and *Tr. dimorphon*) in practically all the common laboratory animals and contrary to general practice, their conclusions regarding the efficacy of these drugs were based for the most part on the results obtained in the larger animals.

In American laboratories, comparatively little has been done on the chemotherapy of experimental trypanosomiasis. Rowntree and Abel (18), working with compounds of antimony, used *Tr. brucei*, *Tr. evansi*, and *Tr. dimorphon* infections in rats, rabbits, dogs, and one donkey, while Schamberg, Kolmer, and Raiziss (19) have expressed a preference for rats infected with *Tr. equiperdum*.

These examples will suffice to show something of the way in which different forms of experimental trypanosomiasis have been used in chemotherapeutic investigations. Doubtless each worker has had his own reasons for using one or another of these experimental infections, but, with few exceptions, little space has been devoted to the exposition of these reasons. The definite conception of the clinical significance of experimental infections in larger animals held by Thomas (20) as the basis for deductions from his therapeutic experiments with atoxyl, raised for the first time the importance of these factors in the ultimate determination of therapeutic efficiency. Later Breinl and Nierenstein (13) devoted considerable space to a discussion of the relative merits of the use of rats in chemotherapeutic experiments and expressed the opinion that the most conclusive evidence can be obtained with rats as they are very susceptible to infections, their reaction is very constant, and their relapsing time is fairly regular and in direct proportion to the trypanocidal action of different com-

pounds. As regards the other animals, they cited the facts that guinea pigs often died after a short course of treatment from no apparent reason and that in the chronic infection of rabbits, parasites were usually present in exceedingly small numbers and the clinical symptoms were generally easily controlled by the trypanocidal drugs. Further, since mice tolerate, in proportion to the body weight, immense doses of different drugs, they considered it unwise to draw general conclusions from mice experiments for the use of drugs in the treatment of sleeping sickness and animal trypanosomiasis. Finally, Uhlenhuth, Hübener, and Woithe (21) pointed out the difference between such infections as those of mice and rats, in which the disease is largely a blood infection, and that of rabbits, in which tissue involvement is the conspicuous feature, but apparently made little use of this fundamental principle.

Taken as a whole, mice have been much more extensively used than any other animal and the infecting organism most commonly employed has been *Tr. brucei*. Next in frequency have come rats, while guinea pigs, rabbits, and other animals have been used in small numbers and largely to supplement mouse or rat experiments. Much the same condition has prevailed as regards the use of different species of trypanosomes. As the matter now stands, this diversity of usage is very disconcerting and might seem to indicate that there is very little involved in the question of the adaptation of experimental trypanosomiasis to chemotherapeutic investigations and that it mattered but little which animal or which organisms were used for the experiments or how they were used. As a matter of fact, this is far from the case and we are inclined to interpret this lack of any concerted plan of procedure as evidence of a too restricted view of the factors involved and, in addition, as a need for a more critical analysis of these factors as a basis for the adaptation of the experimental conditions to the requirements of the investigator. That there are definite requirements to be met may be self-evident, but nowhere do we find a very clear statement of what they may be. Reduced to the simplest terms, the essential requirements of the worker in chemotherapy of trypanosomiasis are a means of determining quickly and accurately the activity of substances upon an infecting organism and a means of determining the curative powers of these substances, which, as will

appear later, may be problems of an entirely different character. The solution of these problems can best be approached through a careful analysis of the various forms of trypanosomiasis including both animals and organisms that may be used, the means of propagating and regulating the infection, and finally, the general response of various species of trypanosomes to therapeutic agents.

## *Experimental Trypanosomiasis.*

### *Propagation of the Infection.*

The propagation of experimental trypanosomiasis may be accomplished in many ways. In principle, the blood of an infected animal which contains the organisms is used as the medium of transference of the infection from animal to animal. This blood, drawn either from a peripheral vessel or from the heart, is diluted with a suitable medium such as isotonic salt solution or a dilute sodium citrate solution and the requisite dose of the infecting suspension is introduced into the next animal, either subcutaneously, intraperitoneally, or intravenously. The general scheme of animal inoculation may be illustrated by a brief description of the methods which we have employed for several years.

For reasons which will appear later, it is advisable to maintain the stock organisms in two classes of animals, one in which the infection is an acute progressive blood infection as in the mouse or the rat, and the other in which the infection pursues a more chronic course such as the guinea pig. Continuous direct passage from animal to animal of the same species is desirable in order to maintain uniformity of infection reactions where the organisms are to be used for therapeutic experiments. In transferring stock strains of trypanosomes, the blood from a guinea pig showing a well developed infection (microscopic examination of the blood) with actively motile and non-agglomerated trypanosomes is taken from the ear vein or heart with sterile precautions and diluted with sterile normal salt solution until one sees only one trypanosome in every five or six microscopic fields using the high dry objective. We arbitrarily designate such a suspension as +. Varying amounts of a + suspension (1, 0.5, 0.25, and 0.1 cc.) are injected intraperitoneally into guinea pigs. The precaution is

taken of inoculating several stock guinea pigs at the same time with different sized doses in order that the animals may not all die at approximately the same time and so make the recovery of the strain uncertain, and furthermore, since there is considerable irregularity in finding parasites in the peripheral blood of guinea pigs, it is convenient to have on hand stock animals with varying grades of infection so that there may be as little delay as possible when one wishes to infect series of animals for experimental purposes.

Stock mice are inoculated intraperitoneally with a similar + suspension of infected blood in sterile normal salt solution. The tail of the infected mouse, one showing a + + + or + + + + infection, is cleansed with alcohol and dried and is then bled directly into a small Petri dish containing sterile normal saline solution. On account of the variation in virulence of different species of trypanosomes, we use various sized inoculating doses in order to maintain comparable grades of infections. With our particular strains, the doses used for mice are 0.2 cc. of a + suspension of *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi* and 0.5 cc. of a similar suspension of *Tr. gambiense* and *Tr. equinum*.

Since it is not practicable to maintain stock strains of trypanosomes in all species of animals which may be used for therapeutic purposes, the proper source of the virus to be used in the inoculation of any given animal may be determined, in general, upon the basis of analogy of infection types; that is, the animals which show an acute, progressive, blood infection should be inoculated, if practicable, from a stock virus in which an infection of the same general type is maintained, and, conversely, animals showing a chronic, cyclic infection should be inoculated from a stock virus where a like infection has been maintained. (The influence of passage upon the general type of infection produced, as well as upon the virulence of the organism in question, will be discussed at greater length in subsequent sections of this paper.) To meet these conditions, mice or rats to be used for therapeutic experiments should be inoculated with trypanosomes from a stock strain carried in mice or rats, while guinea pigs and rabbits may be inoculated from a guinea pig stock. The inoculation of such animals is carried out in the following way.

In making subinoculations from stock mice to series of mice and rats for therapy experiments, a + suspension is prepared in the same

way as for stock inoculation and the same sized inoculating dose is used. If a large series of animals is required, it is convenient to anesthetize the stock mouse and bleed directly from the heart into sterile salt solution. When inoculating experimental series of guinea pigs and rabbits, it is our custom to bleed from the heart, using a stock guinea pig which shows a +++ or ++++ infection, and if a large number of animals are to be inoculated, it is a wise precaution to keep the blood suspension slightly warm. We prefer guinea pigs of 350 to 500 gm. weight for therapeutic experiments and inoculate them intraperitoneally with 0.5 cc. of a + suspension of *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi* and with 1 cc. of *Tr. gambiense* and *Tr. equinum*. Rabbits are infected intravenously with 1 cc. per kilo of body weight of a similar + suspension of *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi* and 1 cc. per kilo of a ++ to +++ suspension of *Tr. gambiense* and *Tr. equinum*. We have found with our strains that intraperitoneal inoculations of mice, rats, and guinea pigs, and intravenous inoculations of rabbits give fairly uniform and as a rule satisfactory results as regards the incubation period, the grade and course of the infection, and the length of life of the animal. Certain variations in these points, regardless of the uniformity of dosage, are not infrequent in the larger animals as will appear later.

*Animals and Organisms.*

Since we have in mice and rats on the one hand, and in guinea pigs and rabbits on the other, two groups of animals in which experimental trypanosomiasis manifests itself as two essentially different types of infection, the chemotherapist is confronted by two distinct problems in the treatment of such infection types and hence a choice of animals offers an opportunity of testing the therapeutic efficacy of a compound both as regards its trypanocidal action in the acute blood infections and its potency or curative power in the chronic tissue infections. In addition to this fundamental factor of the infection type, as exemplified by the animal species, the question of time and expense must be considered. Mice and rats are the cheapest animals, they can usually be obtained in abundance, are easily handled, and the results of treatment of these infections are quickly obtained. Com-

paring the results of treatment of infections in mice and rats where the infection is of the same general order, we have found that while there is little difference as regards ease of demonstration of therapeutic activity in the two animals, mice are usually more easily cured than rats and the results of treatment are more constant and more uniform in character both as regards the absolute unit dose and the ratio of the curative to the tolerable dose. Moreover, the end-result of treatment can be determined much more quickly in mice.

Trypanosomal infection of guinea pigs and rabbits is a chronic disease in which tissue involvement and not blood infection is the fundamental factor. The treatment of such a pathological condition is a manifestly different problem than the treatment of an acute blood infection and is more nearly analogous to the naturally acquired forms of trypanosomiasis in man and animals. However, the extensive use of guinea pigs and rabbits entails a considerable expenditure of time and money, and in our experiments we have not attempted to use them for routine preliminary tests of trypanocidal activity of a given substance but have reserved them for the more detailed study of those substances which have shown such activity against the infection in mice and rats. Other animals, such as monkeys, dogs, horses, donkeys, cattle, sheep, and goats have been used by different workers especially to simulate naturally acquired forms of the disease. The infection in all of these animals tends to be subacute or chronic with more or less tissue involvement, depending on the trypanosome used and the size of the inoculating dose, thus falling into the group to which the rabbit and guinea pig belong, with a possible exception of the dog, which when infected with nagana, for instance, may have an acute, rapidly fatal infection, with the almost constant presence of parasites in the peripheral blood.

As in the selection of animals for chemotherapeutic study, a choice of the species of trypanosomes is of equal importance. This choice should be based on the character of the infection produced in the various animals employed and the general response of the organism to therapeutic agents as far as this is known. In our work we have used five species of pathogenic trypanosomes and from the point of view of the general character of infection produced, they fall into two groups. *Tr. brucei* and *Tr. evansi* are highly virulent for laboratory

animals, producing a relatively acute, rapidly progressive, and usually fatal infection, while organisms of the second group, *Tr. equiperdum*, *Tr. gambiense*, and *Tr. equinum*, are less virulent, causing a more prolonged course of infection from a given inoculating dose which terminates fatally in mice and rabbits and usually so in rats and guinea pigs. It must be borne in mind, however, that different strains of the same species of trypanosome may show wide divergences in virulence and in the general character of the infection produced. This, in part, may be due to the length of time of isolation of the particular strain from its original source, together with the rate and number of passages in the particular species of stock animal, as well as inherent differences in virulence of individual strains of the same species. The factor of resistance or response of the various species of trypanosomes to therapeutic agents will be considered in a later section.

The general character of the infections, such as the incubation period, course, and duration of the infection, as observed by a number of investigators, is recorded in Tables I to VIII, for convenience of comparison.

A complete analysis of the data contained in these tables is impossible within the scope of this paper, but on the surface it is evident that experimental trypanosomiasis is an extremely varied condition not only as to the infections produced by different species of trypanosomes but even to the character of the infections produced by a given species of trypanosome in one and the same animal species. While the full significance of these facts to the chemotherapist can hardly be gathered from a mere statistical compilation, it may be seen that, in as far as the common laboratory animals are concerned, there is a fairly well defined line of cleavage between the acute infections produced by this group of organisms in such animals as mice and rats on the one hand, and the more chronic infections in guinea pigs and rabbits on the other. As we have already suggested, these differences are of fundamental importance, but their chemotherapeutic significance can be made fully apparent only by an elaboration of the clinical characteristics of the infections in the different animal species.

TABLE I.

*Tr. brucei (Nagana).*

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *hrs.* | *days* | | *days* | *days* | | *days* | *days* | | *days* | *days* | | *days* | *days* |
| | | | | | | | | | | | | | | Dogs. | |
| Laveran and Mesnil. | I. P.‡ | 24 | 3 | I. P. | | 2½ | S. C. | 2–4 | 15–30 | I. V. | 2–3 | 10–50 | S. C. | 2–4 | 6–14 |
| | | | | S. C. | 2 | 3½–5½ | I. P. | 2–4 | 15–30* (5–61†) | S. C. | 2–3 | 10–50 | | | |
| | | *days* | | | | | | | | | | | | | |
| Kanthack, Durham, and Plimmer. | | 2 | 13* (8–25†) | | 2 | 12* (6–25†) | | 5–7 | 50* (20–183†) | | 8 | 30* (13–58†) | S. C. | 4–6 | 18* (14–26†) |
| Bradford and Plimmer. | | | 9 | | | 6 | | | | | | | | | |
| Plimmer and Thomson. | | | | | 2–3 | 5½ | | | | | | | | | |
| Seidelin. | | | | | | 8–17 | | | | | | | | | |
| | | | | | | | | | | | | | | | Dogs. |
| Bruce (Ouganda strain). | S. C. | | 10–12 | | | | | | | | | | | | 8–16 |
| | | | *hrs.* | | | | | | | | | *mos.* | | | |
| Roehl ("ferrox" strain). | | 2 | 60–70 | | | | | | | | | 1–3 | | | |
| | | *hrs.* | *days* | | | | | | | | | | | | |
| Browning. | S. C. | 24 | 3 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | Dogs. | |
| | | | | | | *hrs.* | | | | | | *days* | | *hrs.* | |
| Rowntree and Abel. | | | | | | 72–84 | | | | | | 12–20 | I. P. | 48–72 | 5–14 |
| | | | | | | *days* | | | | | | | | | |
| Thomas and Breinl. | | | | | | 4–5§ | S. C. | | 40–45 | | | | | | |
| | | | *hrs.* | | *hrs.* | | | | | | | *mos.* | | | |
| Pearce and Brown. | I. P. | 24 | 60–72 | I. P. | 36–48 | 4–6 | I. P. | 3–7 | 14–28 | I. V. | 7–10 (S.) | 1–3 | | | |

The references for the authors quoted in Tables I to VIII are taken from Laveran and Mesnil (Trypanosomes et trypanosomiases, Paris, 2nd edition, 1912) and the articles specifically mentioned in the bibliography of this paper.

* Average. † Extremes. ‡ In the tables I. P. indicates intraperitoneal; S. C., subcutaneous; and I. V., intravenous. S indicates signs; M. S., marked signs. § Estimated.

TABLE II.

*Tr. evansi* (*Surra*).

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *days* | *days* | | *days* | *days* | | *days* | *days* | | *days* | *wks.* | | *days* | *days* |
| Laveran and Mesnil (Mauritian strain). | S. C. | | $11\frac{1}{2}$ | S. C. | | 11 | S. C. | | 34 | | | 4 | | | Dogs. 28 (Mauritian strain). 12–13 (Indian strain). Goats and sheep. Disease lasts about 5 mos. and usually ends in recovery. |
| Thomas and Breinl (Mauritian strain). | S. C. | $3\frac{3}{4}$–4 | $9\frac{3}{4}$–12 | S. C. | 3–$4\frac{1}{2}$ | 8–$11\frac{1}{2}$ | | 6–8 | 40–120 | I. V. | $3\frac{1}{2}$–$5\frac{1}{2}$ | | S. C. | Dogs. 7–9 | Dogs. 16–35 |
| | I. P. | 3 | 9–11 | I. P. | $2\frac{3}{4}$–$3\frac{1}{2}$ | $7\frac{3}{4}$–$10\frac{1}{2}$ | | | | | | | | | |
| Danysz. | S. C. | | 5 | | | | | | | | 5–7 | *days* 30–50 (M.S.) | | | |
| Plimmer and Bateman. | | | | | | | | | | | | • | | | 14 (dog; weight 20 lbs.). |
| Rowntree and Abel. | | | | | | | | | | | | | | | 12–15 (Indian strain). |
| Pearce and Brown. | I. P. | *hrs.* 24–36 | 4–8 | I. P. | *hrs.* 36–48 | 7–8 | I. P. | | *wks.* 4–7 | I. V. | 7–14 (S.) | *wks.* 3–6 | | | 14–21 (Mauritian strain). |

## TABLE III.

*Tr. equinum (Mal de Caderas).*

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *days* | *days* | | *days* | *days* | | *days* | *days* | | *days* | *days* | | | |
| Laveran and Mesnil. | S. C. | $2\frac{1}{2}$ | 6 | S. C. | 3–4 | $7\frac{1}{2}$ | S. C. | 9 | 84* (29–120†) | S. C. | 4–5 | 33* | Pigs, sheep, goats, and cattle contract the disease after exposure to inoculation, in a very mild form which nearly always ends in recovery. | | |
| | | *hrs.* | | | | | | | | | | | | | |
| | I. P. | 30–40 | 5 | | | | | | | | | | | | |
| Thomas and Breinl. | Mice become infected somewhat earlier than rats and the duration of the disease is slightly shorter. | | | S. C. | $2\frac{3}{4}$–$3\frac{1}{4}$ | 6–8 | | | | | 4-6 | 16–59 | | | |
| | | | | I. P. | 2–$2\frac{1}{2}$ | 6–8 | | | | | | | | | |
| Ehrlich and Shiga. | | 24 | 4–5 | | | | | | | | | *mos.* | | | |
| Voges. | | | | | | | | | | | | 1–3 | | | |
| | | | | | *hrs.* | | | | *wks.* | | | *wks.* | | | |
| Pearce and Brown. | I. P. | 24–48 | 5–8‡ | I. P | 24–48 | 5–8‡§ | I. P. | | 4–8§ | I. V. | 7–14 (S.) | 3‖ | | | |

* Average. † Extremes. ‡ Occasionally 10–14 days. § Occasionally spontaneous recovery. ‖ Heavy infection.

TABLE IV.

*Tr. gambiense* (*Sleeping Sickness*).

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *hrs.* | *days* | | *days* | *days* | | | *days* | | *days* | *days* | | | |
| Laveran and Mesnil. | S. C. | | 8* (3–15†) | | | 10‡ | | | 25–291 | | | | | | |
| Monfort. | | | 5 | | | | | | | | | | | | |
| Lafont and Dupont. | | | | | | 12 | | | | | | | | | |
| DeBeurmann, Mouneyrat, and Tanon. | | 24 | 4–5 | | | | | | | | | | | | |
| Danysz. | S. C. | | 6 | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | Dogs. |
| Thomas and Breinl. | | | | | | | | | Wks. or mos.§ | | 5–15 | 128‖ | | | Some died in 3 wks., but majority survived longer, up to 9 mos. Occasional spontaneous recovery. |
| | | | | | | | | | | | | | | | Monkeys and baboons. |
| | | | | | | | | | *mos.* | | | *wks.* | | | Very irregular length of survival, some living over a year. |
| Pearce and Brown. | I. P. | 24–48 | 5–8 | I. P. | 3–4 | 5–11 | I. P. | | 2–6¶ | I. V. | 7–15 (S.) | 8* | | | |

* Average. † Extremes. ‡ 3 mos. with another virus. § Death occurred 4–16 wks. after the appearance of the parasites. ‖ With a chronic mild infection lived 150–273 days. ¶ Occasionally 12 mos.

TABLE V.

*Tr. equiperdum* (*Dourine*).

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *days* | *days* | | *days* | *days* | | | *wks.* | | *days* | *mos.* | | *mos.* | *mos.* |
| | | | | | | | | | | | | | | Dogs. | |
| Laveran and Mesnil (Rouget virus). | | | 6–8 | | | | | | | | | | S. C. Vulva. | 2½ | 9<br>2½ |
| Rouget. | | | | | | 15* | At first guinea pigs were refractory but later virus became virulent for them. | | | | | 1–3–4 | | | |
| | | | | | | | | | | | | *days* | | | |
| Thomas and Breinl. | | | | | | 11 and 18 (2 rats). | 4 animals inoculated with negative results. | | | S. C. | 6–11 | 24–108† | One goat inoculated with negative results. | | |
| Uhlenhuth, Hübener, and Woithe (Ostertag's strain). | I. P. | 6 | 8 | I. P. | 4 | 5 | | | | | | Acut 39–56 days. Chronic, 6 mos. | | | Dogs.<br>*days*<br>48 and 103 (2 dogs).<br>Horse.<br>153 |
| Browning. | | 1–2 | 5–7 | | | | | | | | | | | | |
| | | *hrs.* | | | *hrs.* | | | | | | | *wks.* | | | |
| Pearce and Brown. | I. P. | 24–36 | 4–8 | I. P. | 36–48 | 7–8 | I. P. | | 5–7‡ | I. V. | 10–18 (S.) | 3–6 | | | |

* Some animals refractory to infection. † Some animals alive at 6 mos. ‡ Possibly much longer.

TABLE VI.

*Tr. rhodesiense (Sleeping Sickness).*

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *hrs.* | *days* | | *days* | *days* | | *days* | *days* | | | | | *days* | *days* |
| Monfort. | | | 4 | | | | | | | | | | | | |
| Mesnil and Riegenbach. | | | 4–5 | | | 9* (7–12†) | | | | | | | | | |
| Laveran. | I. P. | 24 | $6\frac{1}{2}$ | S. C. | 2–3 | $8\frac{1}{2}$* (6–18†) | | 8–10 | 41* (19–89†) | | | | | | Dog (1). 15 |
| | S. C. | 48 | | | | | | | | | | | | | Dogs (4). |
| Yorke. | | | | | | | | | | | | | | 4–5 | 9–12 |

* Average. † Extremes.

TABLE VII.

*Tr. dimorphon (Gambian Horse Disease).*

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *days* | *days* | | *days* | *days* | | | *days* | | *das* | *days* | | | *days* |
| Laveran and Mesnil. | | | 8 | | | 23* (10-42†) | | | 24 and 30 (2 guinea pigs.) | S. C. | | 76 and 115 (2 rabbits). | | | |
| Dutton and Todd. | | 2–7 | 16–30 | | 3–12 | 20–70 | | | | | | | | | Dogs. |
| Monfort. | | | 4 | | | | | | | | | | | | 10–19 |
| Thomas and Breinl. | | | 16–130 | | | 18* (7–42†) | | | 13–60 | I. P. | 9* (4-15†) | | | | Young dogs. |
| | | | | | | | | | | I. V. | 4–7 | 26–35‡ | | | 36 |

* Average. † Extremes. ‡ More chronic type, 78–157 days.

TABLE VIII.

*Tr. congolense.*

| Investigators. | Mice. | | | Rats. | | | Guinea pigs. | | | Rabbits. | | | Other animals. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. | Route of inoculation. | Incubation period. | Length of survival. |
| | | *days* | *days* | | | *days* | | *days* | *days* | | | *days* | | *days* | *days* |
| Laveran. | | 8–30 | 105* (18-331††) | | | 19* (15–29†§) | | 7–8 | 14* (9–24†) | | | 27 and 70 (2 rabbits).‖ | S. C. | Dogs. 10–15 | 34* (21–52†) |

* Average. † Extremes. ‡ Usually ends fatally in mice. § Always fatal in rats. ‖ Not always fatal in rabbits.

*Acute Blood Infections of Mice and Rats.*

The experimental infection of pathogenic trypanosomes in mice and rats is, generally speaking, an acute, progressive, and fatal disease characterized by the constant presence of parasites in the peripheral blood. With most strains, the parasites multiply continuously and quite regularly from the time they first appear in the circulation until death, when they seem to be more numerous than the red corpuscles. Signs or symptoms of the infection in these animals are rarely seen up to within a few hours of death. The incubation period, the interval of time between the inoculation and the first appearance of trypanosomes in the peripheral blood, varies in any instance with the species of organism, the size of the inoculating dose, the potency of the virus, and the route of inoculation. With the intraperitoneal and intravenous routes the incubation period is shorter than with the subcutaneous and, generally speaking, the more virulent the species of trypanosome, the shorter is the incubation period. We have used our strain of *Tr. brucei* for all routine work and a description of this infection will serve as a type of the acute blood infections of mice and rats.

Within 24 hours after intraperitoneal inoculation in mice of a small dose of infected blood (0.2 cc. of a + suspension), parasites are found in the circulating blood (+ grade). From the time of their appearance, their number constantly and regularly increases and in 48 hours, the infection is usually of a +++ grade. On the 3rd day (60 to 72 hours), it is ++++ and the parasites approach the number of the red blood corpuscles. With an infection of the virulence and severity of our strain of nagana, mice will die at the end of the 3rd or the beginning of the 4th day. The animals appear perfectly healthy up to the time of death. This may be ushered in by a short period of excitement with tremors, jerking of the limbs, and convulsions, which may be of extreme degree; or the mice may appear drowsy just prior to death and die without convulsions. Rats also, in our experience, may have convulsive attacks before death or may become drowsy during the last hours of life and die without other signs.

The infection produced by *Tr. brucei* in rats is quite similar to that in mice, with the exception that rats live longer than mice after the

number of parasites in the blood has reached a ++++ grade. The incubation period after intraperitoneal inoculation is slightly longer. The rapidity of development of the infection is quite comparable with that observed in mice and a ++++ grade is reached within 3 to 4 days. With such an infection, rats will live from 1 to 2 days, thus dying 4 to 6 days after inoculation.

The infection produced in mice and rats by other species of pathogenic trypanosomes which we have used is comparable with that of *Tr. brucei* as regards the general type of blood infection, although there are certain minor variations which should be considered. Infections with *Tr. equiperdum* and *Tr. evansi* are somewhat less acute than with *Tr. brucei*, mice living from 4 to 8 days and rats from 7 to 8 days. Occasionally the progressive course of a dourine (*Tr. equiperdum*) infection will be interrupted by a day or two in which decreasing numbers of parasites are found in the circulating blood. The incubation period for both *Tr. equiperdum* and *Tr. evansi* in mice is from 24 to 36 hours and in rats is usually slightly longer (approximately 36 hours), after intraperitoneal inoculation. Infections produced with our strains of *Tr. gambiense* are more chronic in character, mice dying in 5 to 8 days and rats in 5 to 11 days. It is uniformly fatal for both species of animals. The incubation period for mice is 24 to 48 hours and for rats usually 48 to 72 hours after intraperitoneal inoculation. With our strain of *Tr. equinum* also, a slower type of infection is produced, mice and rats usually dying in 5 to 8 days with an incubation period of 24 to 48 hours. Occasionally the infection may run a curious relapsing course. On the 4th or 5th day, the parasites in the peripheral blood will greatly diminish in number for 2 to 4 days. At the end of this time, they will recur with increasing rapidity and death will follow in 10 to 14 days after inoculation. We have noted this relapsing type of caderas infection more frequently in mice than in rats, but, on the other hand, we have seen many more caderas mice than rats. Occasionally also, we have seen spontaneous recovery in rats infected comparatively lightly with *Tr. equinum*, but this is not the rule with our strain of this organism. Laveran and Mesnil draw attention to signs and symptoms of approaching death in surra, caderas, and dourine mice which are not usually shown by nagana animals. The mice are very quiet and sit huddled up with

a rough and bristling coat. The animals are insensitive to external stimuli, the eyes are usually closed, and the corneas may become white and opaque just prior to death.

*Chronic Tissue Infections of Guinea Pigs and Rabbits.*

Experimental trypanosomiasis of rabbits, and to a certain extent of guinea pigs also, constitutes a type of infection in which tissue involvement is a conspicuous and predominating feature, and in this respect contrasts sharply with the blood infection of mice and rats. In neither guinea pigs nor rabbits is the presence of parasites in the peripheral blood a constant characteristic of the infection and in rabbits, in our opinion, it is of distinctly minor importance. The visible or external signs of tissue involvement of guinea pigs, it is true, may not be so striking or so constant as they are in the rabbit except in certain chronic infections of long duration. On the other hand, the infection is not of the acute type seen in mice and rats in which the parasitic invasion of the peripheral blood stream is the characteristic and constant feature. Consequently, guinea pigs would seem to be more appropriately classed with rabbits in the group of chronic or subacute tissue infections.

*Trypanosomiasis in Guinea Pigs.*—Guinea pigs inoculated intraperitoneally with our strain of *Tr. brucei* (0.5 cc. of a + suspension) will show a few parasites in the circulating blood within 3 to 7 days. The number of trypanosomes increases slowly for a few days reaching a ++ to +++ grade in about a week after their first appearance, when quite suddenly they disappear from the peripheral blood and one may examine a large number of films without finding a single trypanosome. This condition continues for an irregular period of time, usually 1 to 3 weeks during which, although one may find an occasional organism in blood films, the examinations are usually negative. The parasites invade the peripheral blood stream again as suddenly as they left it and with this second appearance increase rapidly and fairly uniformly in number reaching a +++ to a ++++ grade of infection in about a week. Death usually occurs at this time and is comparatively sudden. It is generally ushered in by local and general muscular tremors, twitching of the limbs, respiratory distress,

general convulsions, and stupor with some spasticity lasting from ½ to several hours. The average length of life of guinea pigs infected with our strain of *Tr. brucei* is 4 weeks, although many have died in 2 to 3 weeks and others have lived 2 months. With a smaller inoculating dose than we usually use and a consequently less acute and more prolonged grade of infection, we have had some nagana guinea pigs which have shown clinical signs of the disease. In these animals, there has been slight loss of hair especially about the eyes and over the back, more or less edema of the external genitalia and anus, together with loss of weight or even emaciation. Such a chronic type of nagana infection, however, is unusual with our strain of *Tr. brucei*, which is quite virulent for guinea pigs. Guinea pigs receiving massive inoculating doses of *Tr. brucei* usually die within a week's time and do not show the characteristic cyclic appearance of parasites in the circulating blood. Such an overwhelming infection is comparable with the acute type seen in mice and rats, although in the case of guinea pigs, the uniformly progressive increase of trypanosomes in the blood may not occur.

Infections in guinea pigs with our strains of *Tr. equiperdum* and *Tr. evansi* are similar in general to *Tr. brucei* infections, although with both of these organisms, the course of the disease is of somewhat longer duration. The characteristic cyclic invasion of the peripheral blood stream by trypanosomes is seen in infections with both *Tr. equiperdum* and *Tr. evansi*, but with *Tr. equiperdum* especially there may be more than one period of remission in which no organisms are found in the blood. With our nagana strain, guinea pigs usually die at the height of the second cycle, or invasion of the peripheral blood, but in *Tr. equiperdum* infections, there is a tendency toward more than two cycles. The succeeding cycles in which one may find parasites are usually shorter than the earlier ones. The length of life of guinea pigs infected with *Tr. equiperdum* is quite irregular but averages from 5 to 7 weeks. Guinea pigs infected with *Tr. evansi* live on an average of 4 to 7 weeks but here again, there is considerable irregularity and definite statements are difficult to make. We have had no spontaneous recoveries with either strain in this species of animal. Chronic infections with *Tr. equiperdum* and with *Tr. evansi* are not rare and may be very prolonged, in which case clinical signs

such as the loss of hair, chronic lesions of the skin, and edema of the genitalia are not infrequently seen, an extreme grade of which is shown by the dourine guinea pig in Figs. 1 and 2. This guinea pig lived 106 days and showed conspicuous signs of the disease, consisting of extensive loss of hair with scaling and thickening of the skin, marked edema of the eyelids, external genitalia, and anus, and a profuse mucopurulent nasal discharge. The animal was extremely weak, thin, and emaciated, with muscular tremors, and blood films showed large numbers of trypanosomes.

Infections in guinea pigs with our strain of *Tr. gambiense* are extremely irregular both as regards the cyclic character of the infection and the length of life of the animal. As a rule, the condition is outspokenly chronic, guinea pigs surviving from 2 to 6 months and sometimes as long as 1 year. The periods of remission in which no parasites are found in the peripheral blood are usually considerably longer than those of guinea pigs infected with other pathogenic trypanosomes. The number of cycles is very irregular and even average estimates are difficult to make. Clinical signs and symptoms may be present in infected animals of long survival, but they are not so prominent or so conspicuous in our experience as in the chronic infections of other trypanosomes. Spontaneous recovery of lightly infected guinea pigs is not uncommon. One such guinea pig in our possession has lived over 4 years and shows no signs of the disease.

Infection of guinea pigs with our strain of *Tr. equinum* is also a chronic condition, similar to that of *Tr. gambiense*, although the average time of survival is somewhat shorter. Guinea pigs usually live from 1 to 2 months, although survivals of 3, 4, and 5 months are not at all uncommon. We have had no instance of spontaneous recovery of guinea pigs with our caderas strain. External tissue involvement is confined, in our experience, to edematous swelling of the scrotum and sheath. The cyclic invasion of the peripheral blood by trypanosomes is a characteristic feature, but as with *Tr. gambiense*, the infection is extremely irregular.

Experimental trypanosomiasis of guinea pigs, therefore, presents certain features which contrast sharply with the infection of mice and rats. There is a characteristic cyclic invasion of the peripheral blood by trypanosomes and in the periods of remission, no parasites are

found. Although the number of trypanosomes increases in the blood during these cycles of blood invasion, this increase is by no means so regular or so uniformly progressive as is usually the rule in the blood infections of mice and rats. While the length of survival of guinea pigs is extremely irregular, generally speaking it is a matter of weeks and months with the ultimate possibility of spontaneous recovery. In addition, in the more chronic infections, clinical signs may appear, all of which is in striking contrast to the infection in mice and rats with the same organisms. These features of the infection in guinea pigs are indicative of two conditions of chemotherapeutic significance: first, the interposing of animal resistance to the progress of the infection, and second, the establishment of true tissue lesions, neither of which, with rare exceptions, is in evidence in either the mouse or rat infection. One of these factors, that of animal resistance, reaches its highest point in the guinea pig; the other, that of tissue involvement and reaction, is most typically developed in the rabbit.

*Trypanosomiasis in the Rabbit.*—Trypanosomiasis in the rabbit as in the guinea pig is an infection of considerable duration but characterized chiefly by the clinical signs and manifestations of disease, while the finding of trypanosomes in the circulating blood plays a distinctly minor part. Thus, in an advanced condition of rabbit trypanosomiasis with pronounced clinical signs and symptoms, no trypanosomes or, at best, only a few may be found in blood films to indicate the existence of an infection. In the majority of the experiments, we have used a strain of *Tr. brucei* carried constantly in stock guinea pigs and the rabbits were inoculated intravenously with 1 cc. of a + blood suspension per kilo of body weight. In these infections the initial signs of the disease usually appeared in from 5 to 7 days and the length of survival of the untreated animals was from 1 to 3 months. More animals died within the first 5 weeks, however, than survived for a longer period. The infection is uniformly fatal in rabbits.

The earliest signs of nagana infection in rabbits appear about the head and external genitalia. Slight puffiness of the upper eyelids, swelling of the lips, reddening and slight thickening of the base of the ears, together with a similar appearance of the external genitalia, may all occur within 1 week after inoculation. Usually, however, only one or two parts of the body are at first involved but within a

few days, new areas are affected. The swelling of one or both upper eyelids with injection of the conjunctival vessels is a common initial sign and may be the only one for several days (Figs. 3 and 4). This condition increases rapidly and the lower lids may also become involved although usually less severely. In a few days, the eyes are not infrequently completely closed (Figs. 5 and 6). By this time, the margins of the lids are considerably reddened, the eyelashes are falling out, and there is a thick yellow exudate gluing the lids together.

Involvement of the base of one or both ears is likewise a characteristic early sign and is easily detected by holding the ear up against the light. Extending upward from the base of the ear, one can see a faint reddening of the tissues with dilatation and congestion of the blood vessels. A day or two later, the base of the ear is distinctly thickened and feels warm to the touch. The swelling rapidly increases both in extent and degree until the ear begins to droop (Figs. 7 and 8), and in a few days it is so swollen and heavy that the animal can no longer hold it erect (Figs. 9 and 10). The hair over the swollen area soon falls out and the skin becomes brawny, rough, and scaly. Crusts and scabs form and not infrequently actual ulceration of tissue occurs (Figs. 11, 13, and 14).

About the time that the eyes are partially closed and the ears beginning to droop, subcutaneous swelling of the face and upper lips appears (Figs. 11 and 12). In some rabbits, however, swelling of one upper lip was the first sign of the disease. At first, these swellings are rather puffy and edematous, but they soon become hard, indurated, and markedly reddened. In the beginning, they are small and focal, the entire lip itself not necessarily being involved but they rapidly increase in size, affecting the entire lip and not uncommonly both lips. A similar development may occur in the facial swellings which may become so marked that the contour from the base of the ear to the nose and lips is an extreme convex curve. The swellings of the face and lips, however, may remain more localized and when the early edema has disappeared, the deeper tissues are seen to be still involved. This is noticeably the case over the bridge of the nose in which swelling and induration of the periosteum with subsequent ulceration is not uncommon. There have also been a few instances of circumscribed swellings toward the end of the nose which are prone

to secondary infection. Accompanying the facial and lip involvement, there is a nasal discharge which usually begins in the 2nd or 3rd week of the infection. This discharge is at first mucoid in character and usually slight in amount but as the disease progresses, it may become very profuse with the formation of thick yellow crusts about the nostrils (Fig. 23).

Involvement of the external genitalia practically always occurs either as an early or late sign of the disease. In the majority of our rabbits, it occurred early and not infrequently was the first sign noted. In male rabbits, slight swelling and reddening of the prepuce or slight enlargement of one or both testicles are the initial signs (Fig. 15). The testicle feels hot, edematous, and somewhat elastic. In a day or two, it may become enlarged to two or three times its normal size (Fig. 16). The scrotum is extremely tense and glistening and the testicle itself becomes markedly resistant and hard. As the involvement advances, the actual swelling usually decreases somewhat but the testicle becomes more indurated and rubbery with a brawny, thickened scrotum (Figs. 17 and 18), which may finally lead to superficial excoriation or even ulceration (Fig. 17). The condition of the prepuce is similar to that of the scrotum and testicles. At first, there is considerable swelling, congestion, and induration (Fig. 19) while in advanced grades of infection, widespread ulceration may occur (Fig. 20). Occasionally, the penis becomes swollen and indurated with but little involvement of the prepuce (Fig. 21), or the prepucial condition may practically clear up to be followed by the enlargement of the penis. In female rabbits, the external genitalia are similarly affected (Fig. 22). The labia are markedly swollen and congested and the vaginal mucous membrane becomes involved in the same process. The anus practically always shows some swelling and induration, although usually this is not a particularly early sign of the infection, but follows the swelling of the external genitalia. Diarrhea is not an uncommon symptom and occasionally a white mucous discharge from the rectum occurs, suggesting the involvement of the intestinal mucosa in the infectious process.

A certain proportion of rabbits showing some or all of the signs of nagana infection described above usually die from 3 to 6 weeks after inoculation. In the last 2 weeks, they become emaciated and much

weakened and eat but little (Fig. 25). The picture of such advanced infections is very characteristic (Figs. 18, 23, and 24). When, however, the development of the infectious process is less rapid, with a consequently longer survival of the animal, a more chronic pathological condition occurs. These rabbits survive 2 to 3 months and show an exaggeration of the local signs and symptoms of the advanced and less chronic disease. The most striking signs in such animals are the loss of hair over large areas of skin and necrosis of both soft and bony tissues (Figs. 26, 27, and 28). Most of the face, for instance, as in Fig. 26, may be entirely denuded of hair. The skin is scaly, is greatly thickened, and has lost its elasticity. It is difficult to pick up. The testicles and prepuce may become extremely indurated and on palpation, one gets the impression of fibrosis of the testicular tissue. In the rabbit shown in Fig. 27, there is a conspicuous deep ulceration over the bridge of the nose. Not only have the soft parts in this area been completely destroyed but the cartilage and bone are beginning to show necrosis. In this particular animal, there was little secondary infection of the ulcerated area but this may occur to a marked degree as shown in Fig. 28. This rabbit was an extreme example of a chronic trypanosomal infection. There was an extensive ulceration of the soft tissue of the entire face, nose, and lips with the formation of thick adherent crusts. The mucopurulent discharge from the nose was especially profuse and foul smelling. In addition to the striking condition of the face, there was also marked involvement of both ears, both upper eyelids, and external genitalia together with great weakness and emaciation. Loss of hair in areas other than the face is a common sign in chronic infections. It is perhaps most frequent on the legs and may extend down to the feet and toes (Figs. 29 and 30). The denuded skin becomes considerably thickened and scaly and superficial ulceration may occur with a slight sticky yellow exudate as well as some bleeding and the subsequent formation of crusts. In a few instances, we have seen a patchy loss of hair on the backs of rabbits.

Rabbits infected with other strains of trypanosomes have shown much the same clinical picture as those infected with *Tr. brucei*. Rabbits infected with comparable doses of our strain of *Tr. gambiense* (1 cc. of a ++ or +++ suspension per kilo of body weight) survived an average of 8 weeks. The first signs of the infection were noted 7

to 15 days after inoculation. With this strain of *Tr. gambiense*, we have been struck with the great frequency and comparative severity of genital involvement. In one series of nineteen rabbits, this was the first sign noted in twelve and it persisted in all of them with relatively slight involvement of the face and head. However, it should be stated that our nagana rabbits greatly outnumber those infected with *Tr. gambiense* and the above observation may not be true for a larger series.

Rabbit infection produced by our strain of *Tr. equinum* is quite comparable with *Tr. brucei* as regards length of survival and clinical signs, but in this connection it should be noted that the inoculating doses of *Tr. equinum* are purposely larger than those of *Tr. brucei*. Our experiments with infections produced by *Tr. equiperdum* and *Tr. evansi* in rabbits are not extensive enough to justify any effort to analyze these infections in detail. As far as our experience has gone, the clinical course of the disease caused by these organisms in rabbits is similar to that of nagana.

These descriptions of trypanosomiasis in laboratory animals are, of course, but type descriptions of the infection as it usually occurs and it should be appreciated that the experimental disease is subject to very marked alteration in part due to "natural" or inherent causes but in part also due to causes subject to the control of the investigator.

### *Some Factors Influencing the Course of the Infection.*

In addition to the animal species as a determining factor in the type of trypanosomal infection, there are certain other factors which may influence the character and termination of the infection of a given organism in any particular animal species. First, the dose of infecting organisms, other things being equal, may be so regulated as to insure within reasonable limits the general character of the infection together with the desired length of life of the inoculated animal. This is comparatively easily obtained in mouse and rat infections with the more virulent organisms and may be quite closely approximated with the less virulent species. With larger animals, in which as we have emphasized, a different type of infection obtains, such exactness is a more difficult task but by regulating the size of the inoculating

dose, one may produce a relatively rapid and acute infection or a more prolonged diseased condition or with the less virulent organisms, especially, a mild grade of infection which may end in spontaneous recovery. To meet the requirements of a more exact dosage, Kolmer (22) has devised a method of counting the trypanosomes in order to inoculate animals with known numbers of organisms. The mode or route of inoculation merely affects the period of incubation, the parasites appearing in the blood or the signs of the infection developing sooner after intraperitoneal or intravenous than after subcutaneous inoculation.

Secondly, the virulence of different species of trypanosomes must be taken into account. Generally speaking, *Tr. brucei* is naturally the most virulent species for the more common laboratory animals. *Tr. dimorphon*, *Tr. congolense*, *Tr. equiperdum*, and *Tr. equinum* are among the less virulent and between these extremes in varying degree are *Tr. evansi*, *Tr. gambiense*, and *Tr. rhodesiense*. In addition, the virulence of various strains of the same species must be considered. Although a freshly isolated strain is usually less virulent for laboratory animals than one that has been carried in stock animals for some time, this may not always be the case. A freshly isolated strain of *Tr. gambiense*, for instance, may be highly virulent for a large number of animals, while another freshly isolated strain may produce a chronic or subacute experimental infection in laboratory animals which may or may not end in spontaneous recovery. Moreover, different strains of the same species may show considerable variation in virulence for a given animal species. With certain strains of *Tr. equiperdum*, for instance, mice and rats are easily infected, while with others, the infecting power for these animals is absent or of a very low grade. Most strains of *Tr. brucei* are highly virulent for mice, killing them in 3 to 4 days, yet the Ouganda strain of Bruce (23), kept in stock mice, killed regularly in 10 to 12 days.

The factor of acquired virulence of a particular strain must be kept in mind as well as its "natural" or inherent virulence. The virulence of any strain is subject to experimental influences as by its "direct" passage in the same animal species or by "crossed" passage in different species as well as by its rate of passage. Thomas and Breinl (17), speaking of *Tr. gambiense*, state that "if a strain be re-

peatedly and quickly run through animals of the same species, it will acquire a certain virulency for such a species, but this continues only so long as the strain is not run through other species of animals." However, according to Laveran (24), who gives the following résumé of his experiments, this augmentation of virulence by continued direct passage is not characteristic for all species of trypanosomes: "For *Tr. evansi* (surra of Mauritius and of Mbori) and for *Tr. gambiense*, virulence is increased following a series of passages in guinea pigs. For *Tr.* of Togoland (nagana), the virulence is diminished and for *Tr. congolense*, the virulence is not varied in spite of the great number of passages." It should be noted, however, that these experiments dealt with the guinea pig, an animal which in our experience may give the most varied results depending upon the uncertain factor of the infection cycle. According to other authors, the virulence of a strain by continued direct passage in the same animal species may be modified by this procedure for other animals as well. The modification may take the form of an increased or of an attenuated virulence. Martini (25) reports experiments with a nagana strain (Togoland) which after passage in mice and rats was increased in virulence not only for mice and rats but for dogs, while on the other hand, the viruses of passage through Equidæ, rabbits, and guinea pigs were very slightly virulent for rats, mice, and dogs. Laveran and Mesnil (26) cite the case also of a strain of *Tr. equiperdum* which at first killed mice regularly in 5 to 10 days. This strain was then kept in stock guinea pigs for 2 to 3 months and at the end of this time transferred back to mice. But it had become so much less virulent for mice that the animals had only the slightest grade of infection which ended in spontaneous recovery and the strain was lost. As a general rule, however, it seems to be the consensus of opinion that for most strains of trypanosomes, continued direct passage in any one species of animal increases the virulence for that particular animal species and that crossed passage, from one animal species to another, usually decreases the virulence. Further, the examples of altered virulence in trypanosome strains cited above are striking illustrations of the influence of animal species, as measured by the infection type, upon the organism in question. In addition, the rate of passage of a given animal species is usually an influencing factor, since the rapid trans-

fer from animal to animal at the height of the infection tends to increase the virulence of the strain.

The viability or infecting power of the virus is still another factor which measurably influences the character and termination of any particular infection in a given animal. The blood used for inoculation should be taken from a live or freshly killed stock animal, since pathogenic trypanosomes soon lose their infective power after the death of the host as has been shown by a number of observers. The stage of the infection of the stock animal should also be considered. In the continually progressive infection of mice and rats, the number of parasites in the blood constantly increases, but immediately before or at the time of death of the animal, a number of the parasites show degenerative changes and loss of motility. After diluting the blood for inoculation, it is apparent that under these conditions inoculating doses, which contain comparatively few parasites, would be extremely irregular as regards their infective power. Moreover, toward the terminal stages of an infection, there are present in the blood certain "immune substances," the nature and amount of which are as yet little understood. If in an endeavor to compensate for the comparatively few infecting organisms in the inoculating dose, one increases either the size or the concentration of the dose, the amount of immune substances is also increased at the same time, so that in any case the infecting power of such blood in proportion to the number of trypanosomes may be considerably diminished. On the other hand, if the blood is taken at an early stage of the infection, when there are comparatively few parasites, it is difficult to make an even suspension and an irregularity in the infection produced will result. If subinoculations are to be made from guinea pigs, the cyclic type of infection, characteristic of this animal species, should be borne in mind. When the infection is at its height, just prior to a blood crisis in which the parasites disappear from the circulating blood, degenerative forms and agglomerations of the organisms are frequent. Obviously, this stage as well as the periods of remissions are not favorable times for subinoculations into series of animals in which regularity of incubation together with uniformity of character and termination of infection is of importance.

*Resistance of Specific Organisms to Therapeutic Agents.*

Another set of conditions which the chemotherapist must consider is introduced by the factors of specific and strain resistance of trypanosomes to therapeutic agents as far as these facts are known. There have been numerous examples of such resistance of different species to a particular therapeutic agent reported in the literature. In his first experiments on the therapy of experimental trypanosomiasis, Ehrlich noted that trypan red was less active in nagana than in caderas mice. Atoxyl, which has a marked action on *Tr. gambiense* infections according to Mesnil, Nicolle, and Aubert, acts only feebly on *Tr. dimorphon* (Wenyon, Laveran) and has no action whatever on *Tr. congolense* (Laveran) (27). Monfort (28), in treating infected mice with arsenophenylglycine, found from the point of view of relapses that *Tr. gambiense* infections were the most easily cured, *Tr. rhodesiense* followed, *Tr. dimorphon* were only fairly easy, while *Tr. congolense* infections were highly refractory to the drug, sterilization being only of short duration. Laveran (29), who studied the treatment of *Tr. dimorphon* and *Tr. congolense* infections in guinea pigs with arsenic trisulfide, noted that this compound which had but little action against the first infection was very efficacious against the second. Mesnil and Brimont (30) state that surra and dourine infections were less resistant to tartar emetic than were nagana, caderas, *Tr. gambiense*, and *Tr. dimorphon* infections. Danysz (31) found that *Tr. rhodesiense* infection in mice was more resistant to 88[2] than *Tr. evansi*, and in order to obtain the same curative result, it was necessary to use twice as large a dose. More recently, Yakimoff and Wassilevsky (12) found, in working with dourine infections in mice, that the ratio of the curative to the tolerable dose of 102[1] (luargol) was 1:3, while Danysz reported the striking ratios of 1:80 in surra mice and 1:100 in mice infected with *Tr. gambiense*. In general, with our five strains, we have found that *Tr. equinum* is the least resistant and that *Tr. gambiense*, *Tr. brucei*, *Tr. evansi*, and *Tr. equiperdum* follow in the order named.

The differentiation between the virulence and the resistance of species of trypanosomes should be borne in mind since a marked resistance to therapeutic agents is not necessarily accompanied by a

high degree of virulence and *vice versa*. The classical example of *Tr. lewisi* may be cited as an organism of extremely low virulence but of high resistance to all known therapeutic agents. Furthermore, it should be emphasized that the resistance of the various strains of a species of trypanosome is not constant and that the widest differences in the resistance of different strains may be encountered.

Finally, as Laveran and Mesnil and Ehrlich have pointed out, one cannot conclude from the therapeutic results obtained against a particular infection in one species of animals what the results will be against the same infection in another animal species. In our experience, both *Tr. evansi* and *Tr. equiperdum* are more easily dealt with in rats than in mice, a condition the reverse of which usually obtains.[1]

*Principles of Adaptation and Utilization; Conclusions.*

We have endeavored to show that the use of experimental trypanosomiasis in chemotherapeutic experiments should be based upon a clear conception of the experimental disease as it occurs in laboratory animals and that the use of one or another of the experimental infections is not to be undertaken in a haphazard fashion but that there are definite principles upon which the experiments may be based.

These considerations may appear obvious, but as one searches through reports dealing with experimental chemotherapy of trypanosomiasis, it is impossible to find any clear-cut application, or even recognition of these principles as a whole, to the problem of chemotherapy. Some have emphasized one point and some another, but on the whole, the problem of adaptation has been conceived with a very limited regard for the principles set forth. In the first place, one must know the behavior of the various species of trypanosomes in the animal body, the character of the infection produced, and the response which may be expected from these organisms to the particular

[1] In speaking of drug resistance of different species or strains of trypanosomes, the phenomenon of drug fastness should be called to mind. The differentiation between natural and acquired resistance or fastness to drug action is extremely difficult and since so much experimental work has been done with the various laboratory strains of trypanosomes, it is now almost impossible to say whether the resistance to drug action exhibited by any of these organisms is natural resistance or one which has resulted from laboratory handling.

class of therapeutic agents to be employed, and further than this, one must know even the peculiarities of the particular strain to be used. It is of equal importance also that one should be familiar with the various factors which may influence these reactions and the means to be employed to insure constancy of reaction under all circumstances. Finally, one should realize clearly the limitations to the usefulness of any particular form of the experimental infection or species of trypanosome as well as the limitations to deductions which may be drawn from all classes of experiments.

The animal factor divides experimental trypanosomiasis of laboratory animals into two main classes: the acute blood infections of mice and rats and the chronic tissue infections of guinea pigs and rabbits. From the point of view of the chemotherapy of experimental trypanosomiasis, these two types of infection present totally different problems. Treatment of the acute infections of mice and rats is essentially one of speed, since the duration of the disease is but a matter of days and resolves itself into the treatment of a condition analogous to a bacteremia in which the multiplying parasites are predominatingly found in the circulating blood. A uniform and constant grade of infection is usually easily regulated in mice and rats and since relapses generally occur within 2 weeks after treatment, the more remote therapeutic effects are not delayed. On the other hand, certain factors of importance which are not included in the infection of mice and rats are supplied by trypanosomiasis of guinea pigs and rabbits. In the natural disease of human beings and of animals, tissue involvement is a conspicuous feature and the question of tissue penetrability must be taken into account. In experimental trypanosomiasis of the rabbit and of the guinea pig to a somewhat less extent, tissue involvement with the consequent clinical signs and symptoms predominates and the use of these animals directly supplies the necessary experimental conditions which are wanting in the infections of mice and rats. Not only have the parasites to be killed by the therapeutic agent in treating rabbit trypanosomiasis, but the involved tissue, the site of pathological changes produced by the invading trypanosomes, must be penetrated by the trypanocidal substance, and furthermore, the lesions themselves must be healed. In addition, the factor of duration of the experimental infection must be considered, especially from the

practical outcome of the chemotherapeutic problem, since trypanosomiasis of human beings and of most animals is essentially a chronic rather than an acute disease, lasting weeks, months, and even years. Emphasis should also be laid on the individuality and irregularity of rabbit and guinea pig trypanosomiasis which adds considerably to the value of these animals in any extensive therapeutic experiments. The treatment of infected rabbits especially, resolves itself into the treatment of individuals and such a plan of procedure is directly comparable with that pursued in dealing with the naturally acquired disease. As Strong and Teague (32) have pointed out in their paper on the treatment of surra in the Philippine Islands, the methods of treatment that have given satisfactory results in mice and rats have failed to cure larger animals. In order, therefore, to reproduce, with the usual laboratory facilities, experimental conditions analogous to the natural infections of man and animals, we have utilized the rabbit as the animal that most nearly fulfills the desired requirements. From this point of view, the results of treatment of chronic tissue infections are indicative of curative power or therapeutic potency as contrasted with trypanocidal action.

The selection of the species of trypanosomes should be based on a knowledge of the type and course of the infection produced in the various animal species and on its general resistance to therapeutic agents. The virulence of any particular strain is dependent largely on its inherent natural qualities but may be measurably influenced by certain extraneous factors such as the length of time of isolation and the method of propagation, which are subject to the control of the investigator.

Treatment of experimental trypanosomiasis of mice and rats is largely a matter of speed of action. Since these animals are easily procurable and the results of treatment are quickly seen, the chemotherapist has in this type of infection a valuable experimental test for the quick determination of the therapeutic activity of a compound. For all preliminary routine work, especially if a large number of compounds are to be tested, the utilization of mouse and rat infections gives definite and valuable information in a relatively short space of time. On the other hand, since experimental trypanosomiasis of guinea pigs and rabbits is predominately a chronic tissue

infection more nearly analogous to the naturally acquired forms of the disease, the factor of ultimate curative power or potency of a compound, rather than its immediate trypanocidal action, is emphasized in the treatment of such infections. From the point of view of the chemotherapist, the two types of infections supplement each other and the proper utilization and adaptation of each type constitute a logical basis for procedure in the chemotherapy of experimental trypanosomiasis.

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## EXPLANATION OF PLATES.

### Plate 4.

Fig. 1. *Tr. equiperdum.* Chronic infection. There is loss of hair about the eye and over the nose and right lip. The skin in these areas is thickened and scaly.

Fig. 2. *Tr. equiperdum.* Chronic infection. There is loss of hair over a large area of the back, haunches, and legs with marked thickening and scaling of the skin. The anus, testicles, and prepuce are greatly enlarged and indurated.

### Plate 5.

Fig. 3. *Tr. brucei.* Very early infection. The upper eyelid is slightly swollen and puffy.

Fig. 4. *Tr. brucei.* Early infection. Both upper eyelids are very swollen and the eyes are partially closed. The base of the right ear is similarly involved and the ear is beginning to droop.

### Plate 6.

Fig. 5. *Tr. equinum.* Moderately advanced infection. The eye is partially closed, the face is swollen, crusts are beginning to form over the nose, and the ears are partly drooping.

Fig. 6. *Tr. brucei.* Moderately advanced infection. The eye is completely closed and the face between the eyes and over the bridge of the nose is swollen.

### Plate 7.

Fig. 7. *Tr. brucei.* Early infection. The left ear is swollen and thickened about the base and is hanging down.

Fig. 8. *Tr. brucei.* Moderately advanced infection. The condition of the left ear has increased and the right ear has become involved. Both upper eyelids are swollen and the left eye is partially closed. The face and lips are considerably swollen.

PLATE 8.

FIG. 9. *Tr. brucei.* Early infection. The left ear is slightly swollen and is beginning to droop.

FIG. 10. *Tr. brucei.* Moderately advanced infection. The right ear droops, the right eye is almost closed, there is marked puffiness of the upper eyelid, and the face is swollen.

PLATE 9.

FIG. 11. *Tr. brucei.* Moderately advanced infection. The left lip is swollen and enlarged. The left ear shows a loss of hair with scab formation of the skin along its outer margin.

FIG. 12. *Tr. brucei.* Early infection. The entire face is very much swollen, the lips slightly so. Eyelids and ears are not yet involved.

PLATE 10.

FIG. 13. *Tr. brucei.* Advanced infection. There is loss of hair together with an extensive scab formation along the outer margin of the left ear. Both ears hang down. (The left was held up in order that the picture might be taken.) The left eye is almost closed and there is marked swelling of the face.

FIG. 14. *Tr. brucei.* Moderately advanced infection. There is an extensive scab formation with loss of hair about the base of the left ear extending up along the outer surface and margin. The face over the bridge of the nose is somewhat swollen.

PLATE 11.

FIG. 15. *Tr. brucei.* Early infection. Both testicles are swollen, the left especially so.

FIG. 16. *Tr. gambiense.* Early infection. The right testicle is enormously swollen, the left slightly so. The scrotum is very tense.

FIG. 17. *Tr. gambiense.* The same rabbit as in Fig. 16, 4 days later. The right testicle is decreased somewhat in size and the left is now practically the same size. The scrotum has become indurated and brawny and on the right side, there is a superficial ulcer.

FIG. 18. *Tr. brucei.* Advanced infection. Both testicles are larger than normal and are exceedingly hard. The scrotum has become thickened and indurated. The prepuce is slightly swollen and is indurated.

PLATE 12.

FIG. 19. *Tr. brucei.* Early infection. Moderate swelling and induration of the prepuce.

FIG. 20. *Tr. brucei.* Advanced infection. Extreme swelling, induration, and ulceration of the prepuce. Moderate involvement of the testicles.

FIG. 21. *Tr. brucei.* Advanced infection. Swelling and induration of the penis and right testicle. The left testicle is slightly affected. Prepuce not involved.

FIG. 22. *Tr. brucei.* Moderately advanced infection. Swelling and induration of the vulva and anus.

PLATE 13.

FIG. 23. *Tr. brucei.* Advanced infection. Marked lesions of eyes, ears, face, and nose with thick crusts about nose and lips. There is a profuse purulent discharge.

FIG. 24. *Tr. brucei.* Advanced infection. The face is swollen and indurated and the eyelids are glued together with a purulent exudate. The base of the left ear is affected.

PLATE 14.

FIG. 25. *Tr. brucei.* Advanced infection. Marked general emaciation with few local signs. The eyelids and face are slightly swollen.

PLATE 15.

FIG. 26. *Tr. brucei.* Chronic infection. Loss of hair over face, nose, and about the eyes. The underlying skin is indurated and has lost its normal elasticity. Scab formation about the eyes.

FIG. 27. *Tr. brucei.* Chronic infection. Necrosis of the soft and hard parts over the bridge of the nose. Moderate involvement of the eyelids and base of ears.

FIG. 28. *Tr. brucei.* Long standing, chronic infection. Extreme necrosis of face, nose, and lips with secondary infection. Marked involvement of eyelids and ears.

PLATE 16.

FIG. 29. *Tr. brucei.* Chronic infection. Loss of hair with induration and scaling of the skin along the hind leg.

FIG. 30. *Tr. brucei.* Chronic infection. Loss of hair, induration of the skin, and superficial ulceration with scab formation of the leg.

(Pearce and Brown: Experimental trypanosomiasis.)

(Pearce and Brown: Experimental trypanosomiasis.)

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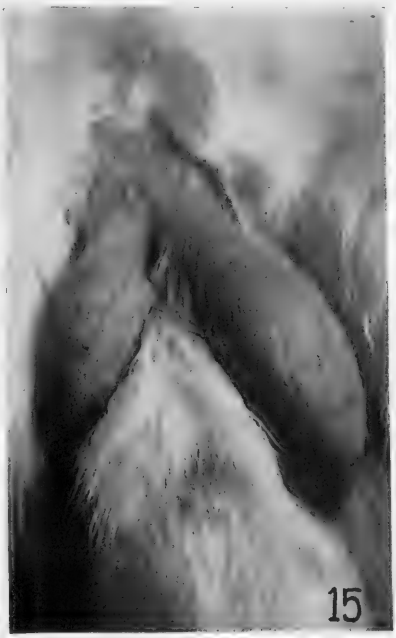

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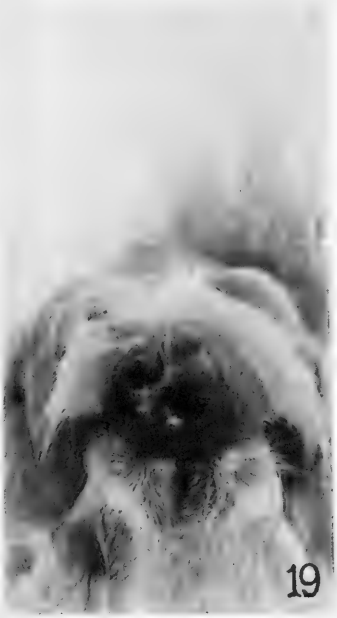

(Pearce and Brown: Experimental trypanosomiasis.)

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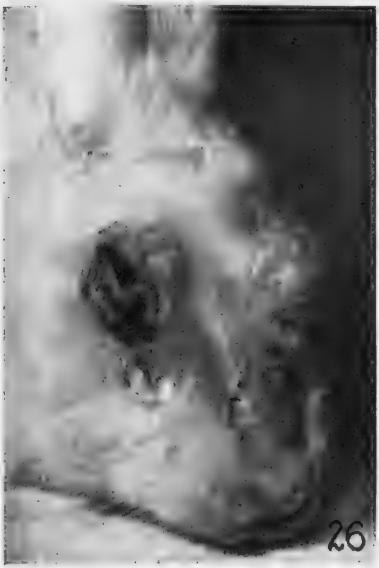

(Pearce and Brown: Experimental trypanosomiasis.)

(Pearce and Brown: Experimental trypanosomiasis.)

# STUDIES IN BOVINE MASTITIS.

## I. Non-Hemolytic Streptococci in Inflammation of the Udder.

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### INTRODUCTION.

Mastitis is one of the more important diseases affecting cows. Not only is it of serious nature economically, but within the past few years it has become of interest from the aspect of public health. Much of the recent work has tended to show that certain streptococci isolated from inflamed udders are closely related to if not identical with those found in certain epidemics of sore throat. Smith and Brown,[1] Davis and Capps,[2] and others believe, however, that these streptococci are of human origin and gain access to the udder through the teat canal.

Nocard and Mollereau[3] appear to have been the first to undertake a study of the etiology of bovine mastitis. They succeeded in isolating streptococci from milk obtained from the udders of ten cows suffering from mammitis. The streptococci are described as growing in long chains in bouillon and staining well with Gram's method. In addition they state that the streptococci fermented sugar media.

Lucet[4] examined milk from twenty-one animals affected with mastitis. From twelve he obtained non-gelatin-liquefying, Gram-negative bacilli; seven were infected with gelatin-liquefying, Gram-positive micrococci. The other two were suffering from streptococcic infection of the udder.

Guillebeau[5] found ten species of organisms associated with infections of the mammary gland. He grouped them as follows: three species of non-gelatin-

[1] Smith, Theobald, and Brown, J. H., *J. Med. Research*, 1914–15, xxxi, 455.

[2] Davis, D. J., and Capps, J. A., *J. Infect. Dis.*, 1914, xv, 135.

[3] Nocard and Mollereau, *Bull. et mém. Soc. centr. méd. vét.*, 1884, 188.

[4] Lucet, *Rec. méd. vét.*, 1889, vi, series 7, 423.

[5] Guillebeau, *Landswirtsch. Jahrb. Schweiz*, 1890, iv.

liquefying micrococci, one group of gelatin-liquefying cocci, two species of streptococci, three kinds of bacilli producing gas in sugar gelatin, and a single strain of rods liquefying gelatin. The results of his bacteriological findings in 85 cases may be given as follows:

| | |
|---|---|
| *Staphylococcus mastitidis* | 33 times. |
| *Galactococcus versicolor* | 12 " |
| " *fulvus* | 5 " |
| " *albus* | 2 " |
| *B. guillebeau* a | 20 " |
| " " b | once. |
| " " c | " |
| *Streptococcus mastitidis sporadicæ* | 8 times. |
| " " *contagiosæ* | 3 " |

Jensen[6] subsequently identified *B. guillebeau* as *B. coli*. In addition he obtained *B. coli communis* and *B. lactis aerogenes* from several cases of garget.

Steiger[7] undertook a detailed study of the disease and in all examined forty-five cases in cows and one in a goat. The summary of the bacteriological findings may be given as follows:

| | |
|---|---|
| *Staphylococcus mastitidis* | 6 cases. |
| Galactococci | 10 " |
| Streptococci | 10 " |
| *B. coli* | 14 " |
| Mixed infections, *B. coli*, streptococci, and *B. necrophorous* | 6 " |

In addition, he had ample opportunities for clinical observation and was able to obtain considerable material for histological study. He discusses the possible modes of infection and considers that the usual mode is through the milk duct. It is admitted, however, that localization of a specific infection in the mammary gland may be caused by metastasis through the blood stream or lymph channels. The possibility of wound infection is also considered.

Unfortunately at the time of Steiger's investigation the differentiation of streptococci by their action upon carbohydrates and hemoglobin had not become a common practice. In describing both the streptococci and micrococci he employed grape and milk sugar. The streptococci were described as having a diameter of 1 micron. They grew in bouillon as diplococci or in short or long chains. Some grew diffusely throughout the medium, others left it clear. In agar they grew as small punctiform colonies. White mice were used to test the pathogenicity of the isolated streptococci. Some strains were highly virulent, others less so, and some produced apparently no ill effect.

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[6] Jensen, C. O., *Ergebn. allg. Path. u. path. Anat.*, 1897, iv, 830.

[7] Steiger, P., *Centr. Bakteriol., 1 te Abt., Orig.*, 1904, xxxv, 326, 467, 574.

Prior to Savage's[8] studies of microorganisms in mastitis, attempts to differentiate many of the organisms by more recent methods had not been made. Many believed that all streptococci isolated from inflamed mammæ were identical. Savage appears to have been the first to undertake a study of the action of bovine streptococci upon carbohydrate media. He observed many strains isolated from the milk of normal cows and from the milk of cows suffering from inflammation of the udder. Inoculations of white mice were resorted to in order to establish pathogenicity. Mention is not made of the action of any strains upon hemoglobin. Examinations of milk from thirty-one cows afflicted with mastitis were recorded as follows:

| | |
|---|---|
| Due to streptococci | 21 cases. |
| " " staphylococci | 5 " |
| " " *B. coli* | 1 case. |
| " " *B. tuberculosis* | 1 " |
| Of doubtful origin | 3 cases. |

Savage did not consider the problem from its economic standpoint but from the relation of the disease to that of the public health. The more important points in the clinical data of the cases were available and his cytological studies were of considerable value.

Henderson[9] examined the secretions from fourteen cases of mastitis. Usually the examinations were made late in the course of the disease. Two were tuberculous, two were of the purulent type, and the remainder were described as parenchymatous. He believed that he was dealing with a mixed infection of streptococci and *B. coli* in twelve of the animals.

Zwick and Weichel[10] succeeded in isolating *B. lactis aerogenes* from nineteen out of twenty-one cases of acute mammitis. From two individuals they obtained *B. paratyphosus*. Inoculation of the mammary glands of goats with these organisms produced severe inflammations of that organ.

Gilruth and Macdonald[11] also reported an outbreak of acute contagious mastitis caused by *B. lactis aerogenes*. They believe that this organism is not usually pathogenic to cattle when inoculated into the blood stream but it may gain entrance into the milk duct and set up serious inflammation of the mammary glands of lactating cows. Recently Ward[12] reports the isolation of *B. pyogenes* from udder lesions. In reviewing the literature on *B. pyogenes* he cites one reference with regard to its etiological relation to a certain type of mammitis.

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[8] Savage, W. G., *Rep. Med. Off. Local 19Gov. Bd. 06–07*, xxxvi, 253; *Rep. Med. Off. Local Gov. Bd. 1907–08*, xxxvii, 359, 425; *Rep. Med. Off. Local Gov. Bd. 1908–09*, xxxviii, p. xxxiii.

[9] Henderson, J., *J. Compt. Path. and Therap.*, 1904, xvii, 24.

[10] Zwick and Weichel, *Arb. k. Gsndhtsamte.*, 1910, xxxiv, 391.

[11] Gilruth, J. A., and Macdonald, N., *Vet. J.*, 1911, lxvii, 217.

[12] Ward, A. R., *J. Bacteriol.*, 1917, ii, 619.

Kitt[13] reviews the literature and classifies the disease etiologically according to whether it is caused by members of the colon group, of the paratyphoid and *enteritidis* groups, or of the groups of staphylococci and streptococci. He states that it is possible to observe many forms such as catarrhal, parenchymatous, and purulent inflammation, abscess formation, sclerosis, and a general rapid necrosis of the mammary tissue. It is pointed out that one type may succeed another, according to the period of lactation and the general resistance of the animal. Like Steiger and others he believes that the teat canal offers a ready method of access into the gland. Mention is also made of infections through the blood and lymph streams. In this connection he refers to Guillebeau and Hess'[14] experiment in which they injected *B. coli* subcutaneously into goats, which was followed by a localization in the mammary gland.

### *Etiological Studies.*

The following studies were undertaken to define more accurately the species of organisms responsible for disorders of the mammary glands of cows, and if possible to lighten the economic burden imposed upon dairying by these affections. In addition, a more complete description of the biological characters of bovine streptococci obtained from inflamed udders seemed desirable for the purpose of assisting those interested in the public health problem of milk-borne epidemics of tonsillitis.

Much of the material has been obtained from a large dairy herd. Mastitis was more or less endemic. During 1916 it was necessary to dispose of 65 cows because of chronic mammitis. In addition to the actual loss from chronic cases, many animals developed milder forms of the disease generally evidenced by flocculi in the milk and inflammation of the gland. Milk from such quarters was discarded and represented an absolute loss. It is interesting to note that during 1916 the number of animals disposed of because of chronic mammitis doubled the number reacting to tuberculin. It has also been possible to obtain clinical data and samples of milk from other sources.

The following routine procedure is used in obtaining milk from inflamed udders. Milk from the affected quarter is drawn directly into a sterile 6 ounce wide mouthed bottle, and except in winter it is

[13] Kitt, T., in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 2nd edition, 1913, vi, 96.

[14] Guillebeau and Hess, cited from Kitt.[13]

iced at once. A note is made of the animal's general condition and the appearance of the involved quarter. The animal's herd number and its location in the various barns are recorded. The milk is usually plated within a few hours in dilutions of 1:10, 1:100, and 1:2,000. Culture medium employed in all initial examinations consists of 1 cc. of defibrinated horse blood and 12 cc. of 2 per cent veal bouillon agar, to which is added the diluted milk, and the whole plated. The effect on hemoglobin is noted at the end of 24 and 48 hours. Readings are always made from deep colonies.

In counting the leukocytes and other cells in milk a modification of the Doane-Buckley[15] method has given satisfactory results. The original method may be described briefly as follows: 10 cc. of milk are poured into a graduated centrifuge tube and centrifuged rapidly for 10 minutes. The fat and supernatant liquid are drawn off down to the 1 cc. mark. A little saturated alcoholic solution of methylene blue is added and mixed. The counts are made with a Thoma-Zeiss blood counter. Campbell[16] modified the technique by washing the sediment several times with distilled water. He considers the stain unnecessary. Campbell's technique proved very satisfactory, although 0.9 per cent salt solution was substituted as a washing fluid. Stained films from the sediment of the centrifuged milk were prepared for microscopic study.

The examination of milk obtained from 81 animals suffering from various forms of mastitis has revealed the following bacterial associations:

| | |
|---|---|
| With non-hemolytic streptococci | 31 |
| " hemolytic " | 17 |
| " mixed hemolytic and non-hemolytic streptococci | 2 |
| " micrococci | 24 |
| " *B. coli* | 2 |
| " pleomorphic Gram-positive rods | 4 |
| " *B. lactis aerogenes* | 1 |
| | 81 |

[15] Doane, C. F., *Maryland Agric. Exp. Station, Bull. 102*, 1905.

[16] Campbell, H. C., *U. S. Dept. Agric., Bureau of Animal Industry, Bull. 117*, 1909.

Early in the investigation it became apparent that non-hemolytic streptococci were responsible for many udder infections, and it was decided to study several infections of this type. Among these the following cases represent typical spontaneous infections.

### *Case Records.*

*Cow 55.*—Holstein cow, age about 6 years. Said to have had an attack of mastitis in Jan., 1917. Date of parturition unknown. Developed mastitis in the left hind quarter on May 20, 1917.

May 21. First examination of the milk from the affected quarter. The quarter was enlarged, very firm, hot, and painful when manipulated. The walls of the milk duct were thickened. Milk could only be expressed with great difficulty in a very fine stream. A slight rise in temperature was recorded (102.4°F.). The milk was watery and contained many irregular flattened, white flocculi.

Agar plates prepared from milk of the affected quarter revealed 3,600 non-hemolytic streptococci per cubic centimeter. Attempts to ascertain the number of cells failed because they clumped and formed an unbreakable viscid mass after centrifugation. Examination of films from the milk sediment revealed streptococci in chains up to nine cocci and great masses of polymorphonuclear leukocytes. The other quarters appeared normal.

May 23. The milk was less watery but contained many fine flocculi in suspension. It contained 16,950,000 cells per cubic centimeter and the plates revealed 47,000 streptococci per cubic centimeter.

May 25. The quarter was still firm but not feverish or painful. Milk was watery and contained very little fat; the flocculi were numerous. The animal's temperature was normal (101.4°F.).

Left hind quarter:
Cells 16,800,000 per cubic centimeter.
Colonies 5,000 " " " pure culture of non-hemolytic streptococci.

Right hind quarter:
Cells 185,000 per cubic centimeter.
Colonies 50 " " " no streptococci. The milk appeared normal.

Left fore quarter:
Cells 90,000 per cubic centimeter.
Colonies 510 " " " no streptococci.

Right fore quarter:
Cells 460,000 per cubic centimeter.
Colonies 2,100 " " " 95 per cent of the colonies were similar to those observed in plates from left hind quarter.
Films from sediment contained polymorphonuclear leukocytes and a few streptococci. The quarter appeared normal. The milk failed to show flakes.

Examinations were made at usually 3 day intervals, but as they failed to show marked differences the results of many will be omitted. It is interesting to note, however, that on May 28 the milk from the left hind quarter revealed 24,800,000 cells and 723,000 streptococci per cubic centimeter. The quarter had a tendency to become less firm from day to day and on June 8 the corded portion was confined to the lower half of the quadrant. The cells had fallen to 14,200,000 and the streptococci to 250 per cubic centimeter.

June 5. The blood serum of this cow in a dilution of 1:500 completely agglutinated cultures of streptococci from the affected quarter The highest agglutination recorded was on Aug. 1 when the streptococcic suspensions were partially clumped in a dilution of 1: 2,000.

June 8.
Left hind quarter:
Cells 5,300,000 per cubic centimeter.
Colonies 364,000 " " " pure culture of streptococci.
The quarter was still firm. The milk was less watery but still contained flocculi.
Right hind quarter:
Cells 110,000 per cubic centimeter.
Colonies 120 " " " no streptococci.
Left fore quarter:
Cells 110,000 per cubic centimeter.
Colonies 180 " " " no streptococci.
Right fore quarter:
Cells 1,620,000 per cubic centimeter.
Colonies 14,600 " " " pure culture of non-hemolytic streptococci.
The quarter failed to show inflammatory changes and the milk appeared normal.

This animal was under constant observation until July 12. The original swelling of the quarter had contracted into an irregular nodule about the milk cystern. From June 8 until July 12 the elimination of streptococci from the diseased quarter had been very irregular, once falling as low as 8,000 per cubic centimeter. A maximum cell count of 91,000,000 was recorded, although at one time the cells fell as low as 8,800,000 per cubic centimeter. The right fore quarter continued to harbor streptococci.

July 12.
Left hind quarter:
Cells 53,000,000 per cubic centimeter.
Colonies 160,000 " " " pure culture of streptococci.
Right hind quarter:
Cells 210,000 per cubic centimeter.
Colonies 460 " " " no streptococci.

Left fore quarter:
Cells 120,000 per cubic centimeter.
Colonies 340 " " " no streptococci.
Right fore quarter:
Cells 280,000 per cubic centimeter.
Colonies 16,600 " " " pure culture of streptococci.

It was possible to examine the milk from time to time throughout the period of lactation. The left hind quarter ultimately became smaller than the others. The secretion was greatly diminished. The milk continued to contain flocculi and streptococci could always be recovered from the agar plates. The right fore quarter harbored streptococci throughout the lactation period but never revealed abnormalities. The cow gave birth to a calf in December. Up to Feb. 1, 1918, signs of streptococcic mastitis failed to appear. The subject has been averaging 20 quarts of milk per day. Plates from the left hind and right fore quarter have not revealed the presence of streptococci, since the animal calved.

*Cow 56.*—Holstein heifer, lactating for the first time. The animal calved Dec. 19, 1916. Mastitis developed in the right hind quarter May 21, 1917.

May 23. The animal was slightly depressed and a temperature of 102°F. was recorded. The cow had refused all food; the rumen was impacted. The right hind quarter was tense, feverish, and tender, but not noticeably enlarged. The milk from this quarter was much decreased in amount and was extremely thick. The other quarters appeared normal but the milk flow had decreased.

The exudate from the right hind quarter contained 27,300,000 cells and 5,600 streptococci per cubic centimeter. The cells in the milk from the other quarters were well within normal limits and plates prepared from the milk failed to reveal streptococci.

This animal was under observation for 56 days. The quarter softened somewhat but failed to regain its normal appearance; the milk became less purulent but always contained flocculi composed of casein, fibrin, and leukocytes. The highest cell count was recorded on June 11, when 182,000,000 were noted. The plates on this day revealed 1,300,000 streptococci per cubic centimeter.

The other quarters did not become involved, and streptococci were not observed in the plates.

The cow was slaughtered on July 18 and the udder was obtained for further study. The right hind quarter was firm and a trifle smaller than the others. On section the larger milk ducts were practically filled with yellowish flocculent milk. The parenchymatous tissue was pinkish yellow in color and when freshly cut appeared dry and granular; within a short time milk began to exude from the cut surfaces. There was an increase in interlobular connective tissue. The other quarters appeared normal. Pieces of the involved quarter were fixed in Zenker's fluid. Sections for study were stained with methylene blue and eosin. Examination of these sections revealed considerable degeneration and necrosis of portions of the secreting epithelium. In certain lobules it appeared granular; the

nuclei were shrunken and often forced to one side of the cells. The lumen of such an acinus was usually occluded with milk containing many fat cells and polymorphonuclear leukocytes. The interacinar vessels were engorged with leukocytes and red blood cells. In other lobules the secreting epithelium had been blotted out; the acini appeared indistinct. All that remained of the original structure was the supporting framework, a few necrotic epithelial cells, and dense masses of leukocytes.

The epithelium of many of the small lactiferous ducts stained indistinctly. Much of it was degenerated and infiltrated with leukocytes. Leukocytes and fibrin comprised the contents of the lumen. Many of the larger ducts had suffered severely. Much of their lining epithelium had become necrotic or was badly degenerated. The degenerated portions stained poorly, the nuclei were shrunken, and leukocytes had invaded the epithelial cells. The subepithelial connective tissue contained large numbers of round cells Fibrin, necrotic leukocytes, milk, and microorganisms filled the lumen of the ducts.

Mammary glands of several other cows suffering from infection with non-hemolytic streptococci have been studied. In some the lesions are much more marked than in others. In Cow 66, slaughtered early in the course of the disease, some lobules consisted of purulent masses. In others the central acini were necrotic, while the peripheral had been invaded with dense masses of polymorphonuclear leukocytes. Widespread degenerative changes of the secreting epithelium had occurred. The interlobular connective tissue was congested and edematous.

In the left fore quarter of Cow 72 streptococci were found in the milk 13 days before gross changes in the quarter were observed. 75 days later the animal was slaughtered. On gross examination of this quarter much of the epithelial structures of the upper third of the gland had been replaced by connective tissue. The infiltration had extended downward into the center of the quarter in the form of fibrous strands constricting and blotting out many of the lobules.

Cow 141 had suffered from the same type of infection. The disease was chronic. The principal lesions were confined to the larger milk ducts.

Cow 70 affords an excellent example of a severe type of infection with non-hemolytic streptococci. Both hind quarters and the right fore quarter became so severely involved that the animal was killed. Lesions similar to those found in the sections of the gland of Cow 56 were observed.

The milk from affected quarters often varies in different individuals. Its character is influenced by the stage and severity of the infection. Usually at the onset it is more or less watery in appearance and contains many irregular white flakes of casein, fibrin, and cells. The reaction is slightly alkaline to litmus. It fails to coagulate when boiled. In chronic cases the exudate is usually yellow and less watery. The particles are larger, elongated, and have a tendency to coalesce on standing. The reaction is alkaline. Boiling usually produces a prompt coagulation.

It was possible in one instance to study an early infection. The animal had been under observation for 10 days before mastitis developed in the left fore quarter. The first count, on November 12, revealed 750,000 cells and 5,500 streptococci per cubic centimeter of milk. The quarter appeared normal and the milk was unchanged. 4 days later the streptococci had risen to 86,000 per cubic centimeter. Gross changes could not be detected in the quarter. Mastitis developed clinically on November 25. The count on November 27 revealed 19,000,000 cells and 1,640,000 streptococci per cubic centimeter.

Rühm[17] had noted a similar condition previously. In making routine bacteriological examinations from individual cows he was able to detect streptococci in considerable numbers before clinical signs of inflammation appeared.

Streptococci identical with those responsible for the more severe inflammations may gain access to udders and inflict little or no gross changes in the gland or its secretion. Cow 69 was suffering from an infection of the left fore quarter with *Bacillus lactis aerogenes*. The other quarters were not involved. The right hind quarter became invaded with streptococci. The cell count rose to 610,000 per cubic centimeter at the end of a week and 10,800 non-hemolytic streptococci were noted in a cubic centimeter of milk. 1 week later the cells had risen to 1,000,000. The streptococci fell to 7,300. At the end of 10 days the cell count remained stationary but the streptococci had disappeared. It was only after pouring a considerable quantity of this milk through a sieve having 100 meshes to the inch that flocculi could be detected. Clinical mastitis did not develop in this quarter during the succeeding 10 weeks.

[17] Rühm, G., *Woch. Thierheilk. u. Viehzucht*, 1908, lii, 125.

*Morphological and Biological Characters of Non-Hemolytic Streptococci from Inflamed Udders.*

In Table I the morphological and cultural characters of forty strains of non-hemolytic streptococci obtained from cases of mastitis are recorded. Fermented veal bouillon containing 1 per cent of the various carbohydrates and other substances was used to test the fermentative action of the strains. The initial reaction of the media varied between 0.6 and 0.8 per cent acid to phenolphthalein. Tubes 1.5 cm. in diameter, containing 13 cc. of media were used throughout. The column of liquid varied in height from 6.5 to 7 cm. Titrations were made after an incubation of 5 days at 38°C. The figures under each column denote the net production of acid.

The surface colonies on agar are usually round, delicate, slightly raised, almost transparent, and measure between 1 and 2 mm. in diameter. Occasionally one observes larger, flattened colonies. The deep colonies are tiny, ovoid, or biconvex in appearance. A few strains when grown in horse blood agar plates have produced a narrow, green-tinted zone about the deep colonies.

Morphologically the individuals appear as spherical or slightly elongated cocci. The chain formation varies over a considerable latitude. All stain by Gram's method.

Freshly isolated strains have not proved pathogenic for rabbits weighing between 1,500 and 2,000 gm. 1 cc. of a 24 hour bouillon culture injected intravenously failed to produce symptoms in most instances. Two animals revealed slight irregularities in temperature for a few days subsequent to inoculation but localizations failed to develop. Davis[18] isolated non-hemolytic streptococci from three cases of mastitis. He was able to produce joint localizations in rabbits only after the intravenous injection of the growth from two or more blood agar slant cultures.

From Table I it will be noted that the mastitis streptococci fall into two groups. The larger, composed of thirty-four strains, produces acidity in dextrose, lactose, saccharose, maltose, and salicin. The five individuals of the other group agree as to their general characters except that they fail to act upon salicin. The acid produc-

[18] Davis, D. J., *J. Infect. Dis.*, 1916, xix, 236.

TABLE I.

*Morphological and Biological Characters of Non-Hemolytic Streptococci from Inflamed Udders.*

| Strain No. | Grouping. | Gram's stain. | Growth in bouillon. | Milk. | Production of acid in. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Dextrose. | Lactose. | Saccharose. | Maltose. | Raffinose. | Inulin. | Mannite. | Salicin. |
| | | | | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| C.51C. | L.C.* | + | Turbid. | Firmly coagulated. | 4.8 | 4.0 | 3.7 | 4.0 | 0.0 | 0.0 | 0.1 | 3.9 |
| C.51D. | " | + | " | " " | 4.4 | 4.0 | 3.8 | 3.2 | 0.1 | 0.0 | 0.0 | 4.2 |
| C.55L.H.Q. | M.C. | + | " | " " | 4.2 | 3.7 | 3.6 | 3.9 | 0.2 | 0.1 | 0.0 | 3.3 |
| C.55R.F. | L.C. | + | " | " " | 4.5 | 4.0 | 3.9 | 3.8 | 0.2 | 0.2 | 0.0 | 3.0 |
| C.56R.H. | M.C. | + | " | " " | 4.6 | 3.7 | 3.9 | 4.1 | 0.0 | 0.0 | 0.0 | 3.5 |
| C.65A. | L.C. | + | Clear. | " " | 5.0 | 3.5 | 3.5 | 4.6 | 0.2 | 0.0 | 0.0 | 2.8 |
| C.67D. | M.C. | + | Turbid. | " " | 5.3 | 4.4 | 4.5 | 4.5 | 0.1 | 0.1 | 0.0 | 2.9 |
| C.66 | S.C. | + | " | " " | 3.6 | 3.8 | 3.9 | 3.9 | 0.1 | 0.0 | 0.2 | 3.5 |
| C.70R.H. | L.C. | + | Clear. | " " | 3.8 | 4.0 | 3.7 | 3.1 | 0.0 | 0.2 | 0.1 | 2.6 |
| C.70L.H. | " | + | " | Partially " | 4.1 | 4.1 | 3.7 | 3.7 | 0.0 | 0.0 | 0.0 | 3.5 |
| C.70R.F. | " | + | " | Firmly " | 3.5 | 3.8 | 3.2 | 3.2 | 0.1 | 0.1 | 0.0 | 3.0 |
| C.68R.H. | M.C. | + | Turbid. | " " | 3.9 | 3.5 | 3.2 | 3.4 | 0.1 | 0.0 | 0.1 | 2.8 |
| C.72B. | S.C. | + | Clear. | " " | 4.2 | 3.5 | 3.1 | 3.8 | 0.0 | 0.1 | 0.1 | 3.1 |
| C.141 | " | + | " | Partially " | 4.5 | 4.2 | 3.4 | 2.9 | 0.2 | 0.1 | 0.0 | 4.0 |
| M.L. | M.C. | + | " | " " | 2.2 | 2.7 | 2.5 | 2.1 | 0.0 | 0.0 | 0.1 | 2.0 |
| M.S. | " | + | Turbid. | Firmly " | 5.3 | 3.5 | 4.5 | 2.9 | 0.1 | 0.0 | 0.0 | 3.6 |
| M.U. | " | + | Clear. | Partially " | 3.4 | 3.6 | 3.5 | 3.5 | 0.0 | 0.1 | 0.0 | 3.0 |
| M.X. | " | + | Turbid. | Firmly " | 3.8 | 3.1 | 3.2 | 4.0 | 0.0 | 0.0 | 0.0 | 2.3 |
| M.2 | " | + | Clear. | Partially " | 2.3 | 2.9 | 3.2 | 2.5 | 0.1 | 0.0 | 0.0 | 0.2 |
| M.5 | S.C. | + | " | " " | 2.2 | 2.3 | 2.4 | 2.0 | 0.2 | 0.2 | 0.2 | 0.2 |
| M.8 | " | + | " | Firmly " | 2.7 | 3.0 | 2.6 | 2.3 | 0.1 | 0.1 | 0.1 | 0.1 |
| M.10 | " | + | " | " " | 6.5 | 4.2 | 3.7 | 4.7 | 0.1 | 0.0 | 0.0 | 0.5 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| M.11 | L.C. | + | Turbid. | Firmly coagulated. | 4.9 | 3.8 | 3.8 | 5.0 | 0.0 | 0.0 | 0.0 | 2.9 |
| Abs.4 | M.C. | + | " | " " | 2.3 | 2.6 | 2.3 | 2.0 | 1.5 | 1.1 | 1.5 | 1.2 |
| M.21 | " | + | " | " " | 4.2 | 3.4 | 3.0 | 3.3 | 0.1 | 0.0 | 0.1 | 0.0 |
| M.22 | S.C. | + | " | " " | 4.4 | 4.5 | 4.1 | 4.0 | 0.1 | 0.0 | 0.0 | 2.4 |
| M.25 | M.C. | + | " | " " | 3.3 | 2.9 | 2.4 | 3.0 | 0.1 | 0.1 | 0.1 | 1.5 |
| M.34 | " | + | Clear. | Coagulated on boiling. | 3.0 | 3.3 | 3.3 | 3.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| M.35 | S.C. | + | Turbid. | Firmly coagulated. | 4.9 | 4.4 | 4.1 | 5.0 | 0.0 | 0.0 | 0.0 | 3.9 |
| M.37 | M.C. | + | " | Coagulated on boiling. | 3.3 | 2.5 | 2.8 | 3.3 | 0.0 | 0.0 | 0.0 | 1.9 |
| M.38 | " | + | Clear. | Firmly coagulated. | 4.5 | 3.8 | 3.7 | 4.1 | 0.1 | 0.0 | 0.1 | 3.1 |
| M.40 | S.C. | + | " | " " | 5.6 | 3.8 | 4.3 | 4.9 | 0.1 | 0.1 | 0.0 | 3.1 |
| M.42 | L.C. | + | " | Partially " | 3.9 | 3.7 | 3.3 | 3.1 | 0.0 | 0.0 | 0.1 | 2.6 |
| M.45 | M.C. | + | " | Firmly " | 3.6 | 3.5 | 3.4 | 3.2 | 0.1 | 0.0 | 0.1 | 2.4 |
| M.46 | " | + | " | " " | 3.9 | 3.5 | 3.4 | 3.5 | 0.1 | 0.1 | 0.1 | 2.6 |
| M.58 | " | + | " | " " | 4.0 | 3.6 | 3.6 | 3.4 | 0.0 | 0.1 | 0.0 | 2.6 |
| M.61 | L.C. | + | " | " " | 4.1 | 3.8 | 3.7 | 3.4 | 0.1 | 0.1 | 0.0 | 2.9 |
| M.62 | M.C. | + | Turbid. | " " | 4.2 | 3.6 | 3.6 | 3.5 | 0.0 | 0.0 | 0.1 | 3.4 |
| M.73 | " | + | " | " " | 4.2 | 3.9 | 3.9 | 4.0 | 0.0 | 0.0 | 0.0 | 3.7 |
| M.75 | " | + | " | " " | 4.0 | 3.7 | 3.7 | 3.5 | 0.1 | 0.0 | 0.0 | 3.3 |

* The length of the chains in bouillon has been indicated as follows: S.C., chains of six or eight elements; M.C., threads of ten to twenty cocci; and L.C., chains of more than twenty.

tion of Strains M.5, M.8, and M.2 in dextrose, lactose, saccharose, and maltose bouillon is uniformly lower than that generally recorded. Strain Abs. 4 differs markedly from the others in that it ferments all the carbohydrates, although acid production is lower in raffinose, inulin, mannite, and salicin than in the others. This streptococcus was isolated from a subcutaneous abscess of the udder of a cow.

All streptococci grew well in bouillon, some left the medium clear, others produced a diffuse turbidity. A large majority firmly coagulated milk, others produced only a partial clotting after incubation for 5 days. In two instances the medium appeared unchanged when removed from the incubator, but coagulated promptly on boiling.

Since all strains failed to show major differences in their cultural characters, it seemed well to test their interagglutinability with a serum obtained from the injection of a single strain of streptococci. A nonlactating cow was chosen as an experimental animal. Before commencing the injections the serum of the animal was tested for agglutinins against three typical strains of streptococci. Agglutinations were not observed at dilutions of 1:10. Immunization with killed cultures of Streptococcus C.55 was begun on October 15, 1917. The doses were increased gradually, and when it seemed that a resistance had been established, living cultures were inoculated. On December 12 the serum completely agglutinated the streptococci at a dilution of 1:20,000.

The following method was employed in testing the agglutinating properties of each strain. The growth from 24 hour agar slant cultures was suspended in sterile 1 per cent solution of sodium chloride. Usually the suspensions were agitated with a platinum loop to break up the larger particles. All suspensions were diluted with the salt solution to a uniform density. To each cubic centimeter of this test fluid varying amounts of immune serum were added. Readings were made after incubation for 24 hours at 38°C. A tube containing only the suspension was incubated as a control.

Bovine streptococci usually produce homogeneous suspensions in a 1 per cent solution of sodium chloride and do not tend to precipitate spontaneously during the 24 hour incubation period.

All strains are agglutinated to a greater or less degree by the antiserum produced by immunization with a single species. The non-

TABLE II.

*Agglutination Titer of Non-Hemolytic Streptococci Tested with a Serum Produced by the Immunization of a Cow with a Single Strain.*

| Strain No. | Dilutions. | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1:100 | 1:500 | 1:1,000 | 1:2,000 | 1:5,000 | 1:10,000 | 1:20,000 |
| C.55 | +++* | +++ | +++ | +++ | +++ | +++ | +++ |
| C.55R.F. | +++ | +++ | +++ | ++ | + | − | − |
| C.56R.H. | +++ | +++ | +++ | +++ | + | − | − |
| C.51C. | +++ | +++ | +++ | +++ | ++ | + | − |
| C.65A. | +++ | + | + | − | − | − | − |
| C.67D. | +++ | +++ | +++ | +++ | +++ | + | ? |
| C.66 | +++ | +++ | +++ | +++ | +++ | ++ | + |
| C.70R.H. | +++ | +++ | +++ | ++ | + | − | − |
| C.70L.H. | +++ | +++ | +++ | ++ | ++ | − | − |
| C.70R.F. | +++ | +++ | +++ | +++ | + | ? | − |
| C.72B. | +++ | +++ | +++ | +++ | +++ | ++ | + |
| C.68R.H. | +++ | +++ | +++ | +++ | ++ | − | − |
| C.51D. | +++ | +++ | ++ | + | − | − | − |
| M.L. | +++ | +++ | +++ | +++ | ++ | − | − |
| M.S. | +++ | +++ | +++ | +++ | +++ | + | − |
| M.U. | +++ | +++ | +++ | ++ | + | + | − |
| M.2 | +++ | +++ | +++ | +++ | +++ | ++ | + |
| M.5 | +++ | +++ | +++ | +++ | ++ | + | − |
| M.8 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| M.10 | +++ | +++ | ++ | + | − | − | − |
| M.11 | +++ | +++ | +++ | +++ | +++ | +++ | + |
| Abs.4 | +++ | +++ | +++ | ++ | − | − | − |
| M.21 | +++ | +++ | +++ | +++ | + | − | − |
| M.22 | +++ | +++ | +++ | +++ | ++ | + | − |
| C.141 | +++ | + | − | − | − | − | − |
| M.25 | +++ | +++ | ++ | − | − | − | − |
| M.34 | +++ | +++ | +++ | +++ | +++ | ++ | − |
| M.35 | +++ | +++ | +++ | +++ | +++ | ++ | + |
| M.37 | +++ | +++ | +++ | + | − | − | − |
| M.38 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| M.40 | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| M.45 | +++ | +++ | +++ | +++ | +++ | +++ | ++ |
| M.46 | +++ | +++ | ++ | + | − | − | − |
| M.58 | +++ | +++ | +++ | ++ | + | | − |
| M.61 | +++ | ++ | + | − | − | − | − |
| M.62 | +++ | ++ | + | − | − | − | − |
| M.73 | +++ | +++ | +++ | +++ | + | − | − |
| M.75 | +++ | +++ | + | + | − | − | − |

* Clumping attended by complete clearing of the fluid has been recorded as +++. ++ indicates considerable agglutination without the entire clearing of the fluid. A moderate precipitation has been considered +. A negative reaction has been recorded as −.

salicin-fermenting strains—M.2, M.5, M.8, M.21, and M.34—were agglutinated as readily as those which attacked this substance (Table II). Streptococcus Abs. 4 which differed from all the others was likewise agglutinated at a serum dilution of 1:2,000.

All the streptococci except Strains C.141, M.58, M.61, M.62, M.73, and M.75 were isolated from cases of mastitis occurring on one farm. In each case agglutination occurred at a minimum serum dilution of 1:1,000. Of the six strains from other farms five agglutinated at 1:1,000, the other (Strain C.141) was partially clumped at 1:500. Cultures obtained from the same source as the immunizing strain were uniformly agglutinated at higher dilutions than those obtained elsewhere.

Five strains of hemolytic bovine streptococci isolated from inflamed udders were also tested. Each strain possessed many characters in common with the non-hemolytic types. Usually the only distinguishing difference was their action upon hemoglobin. In no instance were any of them agglutinated at dilutions as low as 1:100.

### *Feeding of Mastitis Milk to a Pig.*

Although freshly isolated strains of non-hemolytic streptococci failed to produce marked effects when inoculated into rabbits, it was considered necessary to test the effect of ingestion of large quantities of these organisms. A young pig weighing 99 pounds was chosen for the experiment. It was fed for 15 days with flocculent milk from Cows 55 and 56. The animal averaged about 2 quarts of purulent milk a day. The diet was augmented with a small amount of grain. Morning and evening temperatures were taken before the feeding was begun and during the experiment. The slight variations recorded were well within normal limits. The animal was under observation for 10 days after the milk feeding was discontinued but failed to show symptoms of any disorder. The pig gained 25 pounds during the experiment.

## DISCUSSION.

Mastitis caused by infections with non-hemolytic streptococci is more prevalent than that caused by other classes of microorganisms. Data point to the extreme severity of these infections. Of the thir-

teen animals under observation for a considerable period but four have recovered. The others have either lost the function of the involved quarters or the disease has progressed to such an extent that they no longer remained profitable as milk producers.

It has been the custom of many investigators to consider the entrance of pathogenic microorganisms into the mammary gland in three ways: (1) metastasis from another disease focus within the body; (2) through wounds; and (3) through the teat canal. Localizations through the blood and lymph vessels occur in tuberculosis and actinomycosis as well as in some other maladies. Wound infection is probably responsible for gangrenous forms of mammitis. The probable mode of infections caused by non-hemolytic streptococci is through the teat canal. The disease is local, usually only one or two quarters are involved, and the general condition of the animal is not markedly affected. The elimination of streptococci several days before symptoms develop also points to entrance through the duct of the teat.

Injury has been considered by many investigators to be an important predisposing factor in udder inflammation. This has not been my experience. If injuries occurred they were of such a minor nature that they escaped detection. If injury plays a major part as a predisposing factor one would expect to find, in many cases, ulceration of the lining membranes of the large ducts and milk cystern and abscesses of the parenchyma. Such lesions have not been observed in the material examined.

It has been difficult to trace infection from one animal to another. On the farm where a large proportion of the material was obtained a "gang" system of milking has been adopted. The milkers are each assigned a cow to milk. The attendant washes his hands after milking each animal, and he is assigned another. In this way each man milks two or three cows irregularly spaced about each barn. This procedure renders the tracing of infections difficult. It seems reasonable to suppose that the extreme irregularity of the occurrence of infection throughout the herd may be explained by the transfer of the virus on the hands of the milkers. Clinical cases are constantly appearing. Cows revealing gross changes in one quarter and harboring streptococci in apparently normal quarters must be considered as dan-

gerous virus reservoirs. Incipient cases eliminate streptococci before symptoms develop. One animal suffered from an udder invasion with streptococci identical in every respect with those obtained from severe inflammations but never developed clinical mastitis. These conditions account at least for the spread of the virus. Contamination of the ends of the teats with feces and vaginal secretions may explain other possible sources of infection.

On one farm visited the incidence of udder inflammations approximated 10 per cent of the cows in one barn. It was customary to milk with a milking machine. The general sanitary conditions were excellent, except that the teat cups were not disinfected or sterilized between the milking of individual cows. The infection was probably spread by the contaminated milk cups.

Definite evidence is lacking to show whether the non-hemolytic streptococci isolated from inflamed mammæ are pathogenic for consumers of milk. Milk-borne epidemics of tonsillitis have been attributed to hemolytic streptococci and up to this time the non-hemolytic forms have not been incriminated, although it must be assumed that non-hemolytic streptococci from inflamed udders gain frequent access to the milk supply. The lack of virulence of these organisms when injected into rabbits and when milk containing enormous numbers of these streptococci was fed to a pig indicates their low pathogenicity for species other than bovines.

## SUMMARY.

It seems clearly established that non-hemolytic streptococci are responsible for a considerable number of cases of bovine mastitis. Of the 81 animals examined, 31 were suffering from infections of this type. The lesions produced in invaded quarters varied from an involvement of only the lining epithelium of the large milk ducts to severe degeneration and necrosis of the secreting epithelium. In one instance a considerable portion of the glandular elements had been replaced with connective tissue.

The streptococci fall into two groups when their action on the various carbohydrates is considered. Thirty-four strains fermented dextrose, lactose, saccharose, maltose, and salicin; five others attacked

the first four sugars but failed to produce acid in salicin. All mastitis streptococci failed to act upon raffinose, inulin, or mannite. One species isolated from a mammary abscess produced acid in all the carbohydrates.

All the strains were agglutinated with an antiserum prepared from one typical strain. The agglutination titer varied over wide limits, although all the streptococci were agglutinated at a dilution of 1: 500. None of the strains inoculated proved pathogenic for rabbits. A pig fed on the milk from two typical cases of mastitis remained well.

Acknowledgment is due to Dr. Ralph B. Little, of this Department, who collected many samples of milk and certain clinical data during the investigation.

# THE CLASSIFICATION OF HEMOLYTIC STREPTOCOCCI.

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In previous work (1) upon the classification of streptococci of the non-hemolytic variety, it was shown that no two of the twenty-eight strains investigated were exactly identical. The complement fixation reaction was the chief test by means of which the relation between the various strains was studied. This result was regarded as indicating a high degree of variability in this type of streptococcus.

The object of the present study was to determine whether the same variability prevailed among streptococci of the hemolytic type. Twenty-eight strains from various pathological sources were studied and their cultural characteristics, power to produce hemolysis of red cells, and behavior in the complement fixation reaction are reported in this paper.

As in the study of non-hemolytic streptococci the chief conclusions as to variability are drawn from a consideration of the complement fixation reactions between these hemolytic strains and their corresponding antisera. On account of the frequency with which these strains showed spontaneous agglutination when grown in plain broth, the agglutination test, which was likewise employed at first, had to be abandoned as a means of differentiation.

## *Methods.*

1. For testing the effect of streptococci on red blood cells dilutions of a 24 hour broth culture were made in a row of small tubes, using plain broth as a diluent. Each tube contained 0.5 cc. of culture dilution, and the doses were graduated in the following manner: the first tube contained 0.5 cc. of culture, the second, 0.25 cc., the third, 0.12 cc., etc. To each tube 0.5 cc. of 5 per cent saline suspension of sheep red blood corpuscles was added. After incubation in

the water bath, at 37°C. for 1 hour, the mixtures were examined to determine whether hemolysis had taken place. Streptococci either (*a*) hemolyze the cells, (*b*) produce methemoglobin in the unhemolyzed cells, or (*c*) have no effect upon the cells. The method is not accurately quantitative but the results are more accurate than are those obtained with the blood agar plate method. Both methods were employed, however, for comparison.

2. Fermentation reactions were made in litmus milk and in media containing lactose, raffinose, inulin, salicin, and mannite as test substances. The media were prepared by adding 1 per cent of the carbohydrate test substance to Hiss serum water. In examining the effect of the streptococci on these carbohydrates a tube of each was inoculated with about 0.2 cc. of actively growing broth cultures and incubated for 10 days.

3. For the animal immunization a rabbit was used for each of the twenty-eight streptococci. Attempts were made at first to immunize these animals by intravenous injections of saline suspensions of killed streptococci at 4 day intervals. Although increasing doses equivalent finally to 50 cc. of broth culture were reached, the serum of these animals, except in a few instances, did not show complement-fixing antibodies, and much time was lost. Neither did a long series of small doses succeed in producing immune bodies in the serum. Finally, freshly killed broth cultures were resorted to. The first dose consisted of an injection of 1 cc. of a broth culture heated at 56°C. for 1 hour, the second, 2 cc., the third, 5 cc., the fourth, 10 cc., the fifth, 20 cc. When this dose was reached a second series of inoculations was begun, commencing again with 1 cc. of a broth culture and increasing the dose in the same manner as was done in the first series. All the injections were made at 4 day intervals. Animals treated by this method responded after six to ten injections by the appearance of complement-fixing bodies in the serum. As soon as a serum showed definite fixation with its own antigen, it was tested on the following day against the other twenty-eight antigens.

4. In the complement fixation reaction the various constituents were used in the following quantities: 0.05 cc. of streptococcus antigen, two units of complement; two units of anti-sheep ambo-

ceptor, and the following amounts of immune serum: 0.1, 0.05, 0.025 cc., etc. The complement-antigen-serum mixtures were made up to 1.5 cc. with saline solution and incubated in the water bath at 37°C. for 1 hour. Sensitized cells prepared by mixing 0.5 cc. of amboceptor dilution and 0.5 cc. of a 5 per cent suspension of cells were added and the tubes again placed in the water bath at 37°C. for 1 hour. At the end of this time the reactions were read.

The antigens were prepared as follows: The washed sediment of a 24 hour broth culture was desiccated *in vacuo*. The addition of absolute alcohol to the suspension of washed culture for the purpose of producing precipitation, which was the method employed in the study of non-hemolytic streptococci, was omitted, since this procedure was found to be unnecessary. After desiccation the sediment was ground into a fine soft powder and weighed. 10 mg. were dissolved in 5 cc. of a 2 per cent antiformin solution in the water bath at 56°C. and the solution was neutralized by using litmus paper as an indicator with 0.1 N sulfuric acid. The free chlorine was liberated by adding one or two drops of 5 per cent sodium thiosulfate. The absence of free chlorine was determined by testing with potassium iodide starch paper. The solution was then made up to 10 cc. with carbolized normal salt solution and centrifugalized. If a sediment appeared it was discarded. 1 cc. of the antigen then represented 1 mg. of dried ground bacterial sediment. This method was strictly adhered to, and thus the antigens used in these experiments represented equivalent amounts of the streptococci from which they were prepared.

## RESULTS.

Table I contains a list of the organisms, their sources, and the chief cultural characteristics as outlined above. The sources of these streptococci include most of the pathological processes in which hemolytic streptococci are known frequently to operate. All are true streptococci as can be seen from their insolubility in bile. The observation of others that hemolytic streptococci are more often round individual cocci in chains, than diplococci in chains such as the non-hemolytic streptococci, was confirmed in the study of these strains.

TABLE I.

*Source and Chief Cultural Characteristics of the Strains Used.*

| Streptococcus. | Source. | Gross appearance in plain broth. | Length of chains in plain broth. | Solubility in bile. | Appearance of colony on blood agar plate. | Effect of colony on blood agar plate. | Effect on suspension of red blood cells. |
|---|---|---|---|---|---|---|---|
| 4 | Abscess. | Turbid. | 10–30 cocci in a chain; many short chains. | Insoluble. | Small round colony. | Clear zone of hemolysis. | Hemolysis; no methemoglobin formed. |
| B30 | Preagonal septicemia; chronic endocarditis. | Nearly clear; soft sediment. | Short chains; 6–10 cocci. | " | " " " | " " " | " " " |
| B31 | Blood culture. Sore throat. | Turbid; pasty sediment. | Many short chains; 6–10 cocci. | " | " " " | " " " | " " " |
| B31a | Metastatic abscess in B31. | " " " | Many short chains. | " | " " " | " " " | " " " |
| 200 | Pus. Tenosynovitis. | Nearly clear; soft sediment. | Moderately long chains; 6–20 cocci. | " | " " " | " " " | " " " |
| 201 | Preagonal septicemia; cirrhosis. | Clear; flocculent sediment. | Long chains; 10–20 cocci. | " | Moist, spreading. | " " " | " " " |
| 202 | Pus. Tenosynovitis. | Turbid; soft sediment. | Short chains; 4–12 cocci. | " | Small round colony. | " " " | " " " |
| 203 | Pus. Cellulitis. | Nearly clear; soft sediment. | Mixed lengths; 4–20 cocci. | " | " " " | " " " | " " " |
| 204 | Spinal fluid. Meningitis. | Clear; flocculent sediment. | Long chains; 10–20 cocci. | " | " " " | " " " | " " " |
| 205 | Throat culture. Sore throat. | Turbid; soft sediment. | Many short chains. | " | " " " | " " " | " " " |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 174 | Blood culture. Sore throat. | Turbid; soft sediment. | Mostly short chains. | Insoluble. | Small round colony. | Clear zone of hemolysis. | Hemolysis; no methemoglobin formed. |
| 175 | Spinal fluid. Meningitis. | " " " | " " " | " | " " " | " " " | " " " |
| 182 | " " " | " " " | " " " | " | Moist, spreading. | " " " | " " " |
| 184 | Blood culture. Puerperal sepsis. | " " " | " " " | " | Checker colony. | " " " | " " " |
| 186 | Pus. Peritonitis. | " " " | " " " | " | Small round colony. | " " " | " " " |
| 189 | Pus. Knee-joint. | " " " | " " " | " | " " " | " " " | " " " |
| 190 | Pus. Mastoiditis. | " " " | " " " | " | " " " | " " " | " " " |
| 196 | Blood culture. Scarlet fever. | Clear; flocculent sediment. | Long chains; 30–40 cocci. | " | " " " | " " " | " " " |
| A96 | Postmortem blood culture. Erysipelas. | " " " | Long chains. | " | " " " | " " " | " " " |
| C6 | Pus. Pelvic abscess. | " " " | " " | " | " " " | " " " | " " " |
| C7 | Blood culture. Septicemia. | Turbid; flocculent sediment. | Short chains and clumps. | " | " " " | " " " | " " " |
| CK | Pus. Pharyngeal abscess. | Clear; flocculent sediment. | " " " | | | | |

TABLE I—*Concluded*

| Strepto-coccus. | Source. | Gross appearance in plain broth. | Length of chains in plain broth. | Solubility in bile. | Appearance of colony on blood agar plate. | Effect of colony on blood agar plate. | Effect on suspension of red blood cells. |
|---|---|---|---|---|---|---|---|
| G | Pus. Purulent pericarditis. | Clear; flocculent sediment. | Short chains and clumps. | Insoluble. | Small round colony. | Clear zone of hemolysis. | Hemolysis; no methemoglobin formed. |
| MC | Blood culture. Septicemia. | " " " | " " " | " | " " " | " " " | " " " |
| D10 | Blood culture. Mastoiditis. | " " " | " " " | " | " " " | " " " | " " " |
| D14 | Blood culture. Septicemia. | Turbid; flocculent sediment. | " " " | " | Both small round and moist, spreading colony. | " " " | " " " |
| βad | β-hemolytic types of Smith and Brown. | Nearly clear; soft sediment. | " " " | " | Small round colony. | " " " | " " " |
| β40 | | " " " | " " " | " | Flat colonies.<br>Checker "<br>Ring " | " " " | " " " |

All the strains caused hemolysis of red cells and produced a perfectly clear zone around their colonies on blood agar plates. Colony formation varied, apparently due to the condition of the medium, and both round and so called "checker" colonies were seen to grow from the transplant of a single round colony. Colonies also varied in their capacity to produce hemolysis on blood agar. Often a strain would produce more hemolysis when growing in the depths of the medium than when growing on the surface. A single strain might produce a zone of marked clearing around the colony at one time and a zone which showed scarcely any clearing at another.

Another feature of hemolytic streptococci is their occasional lack of Gram positiveness. This tendency to become Gram-negative was noticed in non-hemolytic strains, after many mouse passages, in an attempt to increase virulence. But the condition is frequently met with in hemolytic strains. The cause of this could not be determined.

Spontaneous agglutination in broth cultures was frequent in this series. Some of the strains which were transplanted many times gradually lost this characteristic and grew with greater turbidity, resembling non-hemolytic strains in this respect.

Only a few of the strains were tested as to their virulence and it was found that this could be raised by repeated passages through animals so that 0.0001 cc. of an 18 hour broth culture killed a white mouse in 24 hours. The experience of many workers has shown that the virulence of hemolytic streptococci can be raised to a high degree. The large number of mice required to determine the virulence of so many strains made it inadvisable to pay further attention to this point which has already been well established.

The fermentation reactions of these strains are strikingly limited and quite uniform (Table II). Although all the strains do not ferment the same test substances there is a noticeable uniformity in their action. In general it can be said that as a class they are weak fermenters compared with the non-hemolytic streptococci. The time necessary to produce acid and a clot in milk, for instance, is seldom less than 48 hours, and it usually requires longer, whereas most of the non-hemolytic strains produce this effect in 24 hours. None of the strains fermented raffinose or inulin. Only six failed to ferment salicin. Four fermented mannite; six fermented only milk

TABLE II.

*Fermentation Reactions.*

| Streptococcus. | Effect on. | | | | | |
|---|---|---|---|---|---|---|
| | Milk. | Lactose. | Raffinose. | Inulin. | Salicin. | Mannite. |
| 4 | ± | + | − | − | − | − |
| 205 | + | + | − | − | − | − |
| 174 | + | + | − | − | − | − |
| 175 | ℁ | + | − | − | − | − |
| 189 | + | + | − | − | − | − |
| MC | + | + | − | − | − | − |
| B30 | + | ± | − | − | ± | − |
| B31 | + | + | − | − | + | − |
| B31a | + | + | − | − | + | − |
| 201 | + | + | − | − | + | − |
| 202 | + | + | − | − | + | − |
| 203 | + | + | − | − | + | − |
| 204 | + | + | − | − | + | − |
| 182 | + | + | − | − | + | − |
| 184 | ℁ | + | − | − | + | − |
| 190 | + | + | − | − | + | − |
| C6 | + | + | − | − | + | − |
| CK | + | + | − | − | + | − |
| G | + | + | − | − | ∓ | − |
| D10 | + | + | − | − | + | − |
| D14 | + | + | − | − | + | − |
| A96 | + | + | − | − | ℁ | − |
| βad | + | + | − | − | + | − |
| β40 | + | + | − | − | + | − |
| 200 | + | + | − | − | + | + |
| 186 | + | + | − | − | + | + |
| 196 | + | + | − | − | + | + |
| C7 | − | + | − | − | + | + |

− indicates no change; +, acid and clot; ∓, acid, partial clot; ±, acid, no clot; ℁, slight acid, slight clot.

and lactose. These results agree, in the main, with those of Lyall (2) who studied 99 strains.

From Table III, which shows the results of the complement fixation reactions, it can be seen that there exists a striking uniformity in the action of this variety of streptococci. The antiserum of each strain gives fixation with the antigens of all the other strains, or, in other words, each strain causes fixation of complement with all the antisera.

TABLE III.

*Results of Complement Fixation Reactions.*

| Rabbit sera immune to streptococcus. | Streptococcus antigens. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C6 | 200 | 202 | 203 | 204 | βad | B30 | B31a | 174 | C7 | D14 | B31 | 201 | CK | MC | G | 186 | 175 | 196 | 189 | β40 | D10 | A96 | 190 | 182 | 4 | 205 | 184 |
| C6 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 200 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 200 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 203 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 204 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| βad | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| B30 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| B31a | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 174 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |

The numbers indicate plus signs, and the reading from above downward indicates the following dilutions: the top figure in each group, a dilution of 0.1 cc.; the second figure, 0.05 cc.; the third, 0.025 cc. Only three readings are given on account of lack of space.

4 indicates no hemolysis; 3, 25 per cent hemolysis; 2, 50 per cent hemolysis; 1, 75 per cent hemolysis; 0, negative reaction or complete hemolysis.

TABLE III—*Continued.*

| Rabbit sera immune to streptococcus. | Streptococcus antigens. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C6 | 200 | 202 | 203 | 204 | βad | B30 | B31a | 174 | C7 | D14 | B31 | 201 | CK | MC | G | 186 | 175 | 196 | 189 | β40 | D10 | A96 | 190 | 182 | 4 | 205 | 184 |
| C7 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| D14 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| B31 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| 201 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| CK | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 3 | 1 | 3 | 4 | 3 | 4 | 2 | 4 | 4 | 3 | 4 | 0 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| MC | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 3 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| G | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 4 | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 3 | 3 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 |
| 186 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 |
| | 2 | 3 | 3 | 3 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 2 |
| 175 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 2 | 3 | 3 | 3 | 4 | 4 | 2 | 4 | 1 | 3 | 1 | 3 | 4 | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 1 | 1 | 2 | 4 | 3 | 3 | 3 | 2 |
| 196 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 2 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 3 | 4 | 2 | 3 | 4 | 4 | 4 | 4 | 2 | 1 | 1 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 3 | 4 | 3 | 4 |
| 189 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 |
| | 3 | 3 | 1 | 1 | 3 | 4 | 0 | 4 | 0 | 3 | 3 | 1 | 4 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 1 | 3 | 4 | 1 | 3 | 3 | 1 |

TABLE III—*Concluded.*

| Rabbit sera immune to streptococcus. | Streptococcus antigens. | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C6 | 200 | 202 | 203 | 204 | βad | B30 | B31a | 174 | C7 | D14 | B31 | 201 | CK | MC | G | 186 | 175 | 196 | 189 | β40 | D10 | A96 | 190 | 182 | 4 | 205 | 184 | | | |
| β40 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| D10 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | | | |
| A96 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 1 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| 190 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 2 | | | |
| | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 2 | | | |
| | 1 | 3 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 3 | 1 | 3 | 4 | 3 | 4 | 3 | 4 | 4 | 3 | 3 | 1 | 2 | 3 | 4 | 4 | 4 | 4 | 2 | | | |
| 182 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 1 | 0 | 1 | 0 | 4 | 4 | 4 | 4 | 4 | 2 | 0 | 0 | 3 | 1 | 4 | 1 | 4 | 4 | 4 | 4 | 1 | 3 | 4 | 4 | 4 | 3 | 4 | 2 | | | |
| 4 | 4 | 2 | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 3 | 3 | 2 | 4 | 4 | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| | 4 | 2 | 4 | 2 | 2 | 4 | 4 | 3 | 4 | 3 | 3 | 2 | 4 | 4 | 4 | 2 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 3 | | | |
| | 3 | 1 | 3 | 0 | 1 | 3 | 3 | 2 | 3 | 3 | 1 | 1 | 3 | 3 | 3 | 0 | 3 | 0 | 3 | 3 | 3 | 1 | 3 | 3 | 3 | 3 | 0 | 2 | | | |
| 205 | 4 | 3 | 1 | 1 | 4 | 4 | 3 | 4 | 1 | 4 | 1 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 1 | 2 | 4 | 4 | 1 | 4 | 4 | 1 | | | |
| | 4 | 3 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | | | |
| | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 0 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | | | |
| 184 | 3 | 1 | 4 | 1 | 1 | 2 | 4 | 3 | 2 | 1 | 0 | 0 | 1 | 4 | 4 | 0 | 3 | 3 | 4 | 0 | 4 | 1 | 4 | 4 | 4 | 4 | 0 | 4 | | | |
| | 4 | 1 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 1 | 0 | 4 | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | | | |
| | 3 | 3 | 4 | 1 | 3 | 4 | 4 | 3 | 4 | 4 | 4 | 0 | 3 | 4 | 4 | 2 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | | | |

There are slight variations. Thus Serum 184 does not give fixation with Strain B31, and in other instances, the reactions were very weak. Whether this irregularity in results was due to deteriorated reagents could not be determined on account of the limited time available for completing the work. The antigens often lost their fixing power after they had been kept for some time and it was therefore frequently necessary to make fresh antigens. The sera of fourteen of the rabbits used were tested before inoculation, and in no case was there evidence of the non-specific fixation sometimes ascribed to normal rabbit serum.

## DISCUSSION.

The strains of streptococci which form the basis of this study, while limited in number to twenty-eight, can be said to represent fairly the hemolytic variety. The strains were derived from the pathological processes usually associated with infection by this variety of streptococcus. The several variations in fermentative activity noted by others were present in this series. Variations in colony formation were observed which do not seem to be fundamental but apparently depend upon such factors as changing conditions of the culture medium. Variations in the clear zone produced about a colony occurred but seem to represent transitory differences in the degree of hemolytic activity, rather than a constant characteristic of the particular strain of streptococcus. The differences in growth in plain broth were not constant. There was a tendency on the part of the strains which showed spontaneous agglutination in their early transplants after isolation to grow with more turbidity after repeated transplantation. This feature was previously noticed in the case of non-hemolytic strains. The fermentation reactions of this group of streptococci, while displaying differences, revealed the fact that the members of this variety of streptococcus were weak fermenters, because two of the test substances, inulin and raffinose, were not fermented by any of the strains.

The differences noted above were of minor significance. On the other hand, the similarity between all the strains studied was strikingly emphasized by the complement fixation reactions. Although the strains came from different pathological sources and displayed many superficial variations in cultural activity, judged by the complement fixation test all the strains were nearly identical.

## CONCLUSIONS.

1. The hemolytic variety of streptococcus is homogeneous, consisting of members that are nearly identical.

2. This homogeneity is most strikingly displayed in the behavior of these streptococci in the complement fixation reaction, all the strains studied reacting in a nearly identical way with all the antisera.

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# THE RELATION BETWEEN HEMOLYTIC AND NON-HEMOLYTIC STREPTOCOCCI, AND ITS POSSIBLE SIGNIFICANCE.

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If streptococci are separated into those which produce hemolysis and those which do not, it will be found that each of these two groups follows definite and different lines of classification. Of the various means of effecting a classification, the complement fixation reaction has proven the most satisfactory.

Study (1) of the non-hemolytic group by this method has shown that these streptococci are not uniform in character. This variability is due to the fact that there are two opposite elements or features in this group, and the position in the group, or individuality of any one member depends upon whether it partakes more of one or the other of these features. This variation is orderly and simply for convenience one of these opposite elements is called the right side, and the other, the left side of the group. On the other hand, study (2) of the hemolytic group, using the same criterion, has shown that all the hemolytic streptococci studied were nearly identical, the variation being too slight to measure by the complement fixation reaction. If these two studies are correct and the same orderly miscibility of characters prevails among streptococci as among other species, then, first, the two varieties should be related, and, second, since a unique element cannot be related in the same way to two opposites, the hemolytic variety should show a relation to either the right side or the left side of the non-hemolytic group. It was the purpose of the present study to answer this question and at the same time to compare the two varieties of streptococci in other ways.

The power to hemolyze red blood cells has been regarded as the feature which divides streptococci into two groups. This division was made possible largely through the work of Schottmüller (3), who by noting the effect of streptococcus colonies on blood agar was able to separate those which produce clearing about the colony, from those which either produce no effect, or produce a greenish discoloration. The term *viridans*, which is descriptive only of this last group, is often incorrectly applied to the whole non-hemolytic group. No descriptive term has been given to the streptococci which produce no effect on red blood cells, other than the term indifferent. In the present study these indifferent streptococci and the so called *Streptococcus viridans* have been included in the same group, called non-hemolytic streptococci. This capacity to produce hemolysis is of fundamental importance because the streptococci which possess this feature stand in striking contrast to those which do not. These points of difference embrace certain features which seem inconstant and of doubtful importance, and others which are constant and probably very important. Of the former class, there are differences in morphology, staining properties, growth in plain broth, solubility, and carbohydrate reactions; of the latter class, differences in immunizing power, in frequency of occurrence in the human body, in power to invade and produce disease, and finally, in immunological classification. The evidence on most of these points is derived from previous studies (1, 2) of twenty-eight strains of each group of streptococcus as well as from the work of others, which will be referred to later.

*Differences in Morphology.*—One difference in morphology already noted by others is the tendency of hemolytic streptococci to maintain their coccoid shape while the non-hemolytic streptococcus is usually a diplococcus in chains. But a more striking difference is the quite constant appearance of hemolytic streptococci in clumps of small cocci, when growing on blood agar. Such organisms usually show well developed chain formation when transplanted to fluid medium but in cultures on blood agar it is possible to distinguish the chain-forming non-hemolytic diplococcus from the clumps of smaller cocci and diplococci which are hemolytic.

*Differences in Staining Properties.*—In attempting to raise the virulence of certain non-hemolytic streptococci by mouse passage, it was noticed that the strains showed a tendency to become partially decolorized when stained by Gram's method. Under ordinary circumstances non-hemolytic streptococci are strongly Gram-positive. Hemolytic streptococci much more often are only weakly Gram-positive and sometimes strains are encountered which are Gram-negative when growing on blood agar The explanation of this inconstant phenomenon was not determined.

*Differences in Growth in Plain Broth.*—It was found that many more strains of hemolytic streptococci grew in plain broth in the form of a flocculent sediment with clear supernatant fluid than of the non-hemolytic variety. Although this was not a constant phenomenon with any strain and while many strains when first isolated grew in broth with clear supernatant fluid and later with turbidity, still after many subcultures fourteen out of twenty-eight hemolytic strains grew as a flocculent sediment in plain broth, while only three out of twenty-eight non-hemolytic strains grew in broth without turbidity.

*Differences in Solubility.*—The observations on the relative solubility of the two groups were confined to the action of antiformin on these bacteria, in the process of making antigens, as described elsewhere (2). The ease and rapidity with which powdered hemolytic streptococci went into solution in 2 per cent antiformin were in striking contrast to the tenacity with which powdered non-hemolytic streptococci resisted solution under the same conditions. In this respect the hemolytic streptococci resemble pneumococci.

*Differences in Fermentation Reactions.*—A comparison of the fermentation reactions of the two groups showed that the hemolytic variety was eminently a salicin fermenter. Thus twenty-two out of twenty-eight strains fermented this substance, while fourteen out of twenty-eight non-hemolytic strains showed similar activity The non-hemolytic streptococci frequently fermented raffinose and inulin, while the hemolytic streptococci rarely fermented these substances. Lyall (4) studied 99 strains of hemolytic streptococci and found that 75 per cent fermented salicin alone and 8 per cent fermented salicin and mannite. Hopkins and Lang (5),

Kligler (6), and Holman (7) present similar results. In general it can be said that the hemolytic streptococcus is more restricted in its fermentative activity than the non-hemolytic variety, and shows an almost selective tendency to ferment salicin.

*Differences in Immunizing Power.*—A marked difference was noted in the powers of hemolytic and non-hemolytic streptococci to produce immune bodies in rabbits. The method of immunization was the intravenous injection of washed bacteria. After four to six injections with non-hemolytic streptococci, the serum of the rabbits contained large amounts of agglutinating and complement-fixing antibodies. But after several months of similar injections of six different strains of hemolytic streptococci, the serum of the rabbits did not contain antibodies. When, however, the supernatant broth of the culture fluid was injected with the organisms, the appearance of the antibodies in the serum could be detected after six to ten injections of hemolytic streptococci. Neufeld (8) succeeded in immunizing rabbits actively against hemolytic streptococci and the sera of these rabbits contained both agglutinating and protective antibodies. His method was to inject intravenously killed bacteria, and after 10 days small doses of living organisms subcutaneously. The latter injection resulted in inflammatory reactions.

Subcutaneous injection of rabbits with living non-hemolytic streptococci in this study has failed to elicit agglutinating or protective antibodies. The fact that the products of growth activity were necessary to bring about immune bodies in the serum suggests the presence, in the case of the hemolytic streptococcus, of a separable substance such as is described for pneumococci by Dochez (9). In this connection attention must be directed to the fact that Neufeld in making agglutination reactions with hemolytic streptococci used the supernatant fluid of ascitic broth cultures. Even though this fluid contained streptococci there is a possibility that the resultant reactions were largely precipitin reactions. In the present study it was not possible to effect a satisfactory agglutination using whole broth cultures.

*Occurrence in the Human Body.*—Hemolytic streptococci are rarely found in normal throats. This has been found true by Smillie (10) and by Holman. In studying the throat cultures of patients with

acute rheumatic fever and subacute streptococcic endocarditis, hemolytic streptococci were rarely found. Non-hemolytic streptococci, on the other hand, were present in the mouths of normal individuals and we have only observed their absence in cases where frank infection by other bacteria resulted in their temporary exclusion from the cultures. Non-hemolytic streptococci, moreover, were found by Andrewes and Horder (11) to be present even in dust. They apparently live under a wide variety of conditions.

*Differences in Invasive Power.*—This striking difference in distribution between hemolytic and non-hemolytic streptococci runs parallel with the difference in invasive quality of these two varieties. The hemolytic streptococcus has established its position not only as an active invader and producer of disease but also as a cause of epidemics. The numerous milk-borne epidemics studied by Davis (12), Smith and Brown (13), and others were shown to be caused by this type of streptococcus. Certain purulent infections, abscesses, and puerperal sepsis have been ascribed to the agency of this organism. Recently it has played a most important part in the etiology of the bronchopneumonia and empyema following measles. On the other hand, non-hemolytic streptococci have played no proven part as the constant causes of disease. They depend, for their parasitic growth on the previous preparation of a focus of lowered resistance. The term "facultative parasites" used by Andrewes (14) in describing their invasive power is suitable. Even in subacute bacterial endocarditis when this type of streptococcus is found constantly in the blood stream, the growth of this variety of streptococci on a previously injured heart valve seems purely saprophytic. The possibility that certain non-hemolytic strains may be primary invaders of sound tissue is obvious, but emphasis is laid on the fact that in comparison with the hemolytic type the non-hemolytic type is extremely inert.

*Difference in Immunological Classification.*—The difference in classification of these two groups has already been described. The classifications referred to are based on immunological reactions and should, therefore, be of fundamental value. To study the relation between the two groups the same immunological reaction was used. At the time this study was made the sera of fourteen rabbits immunized to hemolytic streptococci were available, while the sera of only

TABLE I.

*Relations between Sera Immune to Hemolytic Strains and Antigens of Representative Non-Hemolytic Strains.*

| Sera immune to streptococcus. | Antigens. | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Left side. | | | | | | | | Right side. | | | | | | | | | Homologous antigen. |
| | MB | A84 | A140 | 38D | A102 | B4 | B23 | A141 | A135 | A30 | A179 | R | A65 | A49 | B39 | B29 | B26 | A4 | |
| B31a | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| | 4 | 3 | 4 | 4 | 4 | 4 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 2 | 3 | 3 | 4 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 0 | 0 | 0 | 4 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| MC | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| | 4 | 3 | 4 | 3 | 4 | 3 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| | 3 | 1 | 3 | 2 | 4 | 1 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| D10 | 4 | 4 | 3 | 3 | 4 | 4 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| | 4 | 4 | 4 | 2 | 4 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 1 | 2 | 3 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| A96 | 4 | 4 | 1 | 2 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 3 | 1 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 2 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 2 | 4 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 196 | 4 | 4 | 1 | 1 | 4 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 4 | 2 | 4 | 2 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 2 | 4 | 1 | 4 | 2 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 0 | 3 | 0 | 3 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| B30 | 4 | 3 | 3 | 3 | 4 | 4 | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 3 | 3 | 3 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 3 | 3 | 1 | 3 | 4 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 1 | 2 | 0 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| β40 | 4 | 2 | 3 | 2 | 4 | 3 | 4 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 2 | 2 | 2 | 3 | 3 | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 1 | 2 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE I—*Concluded.*

| Sera immune to streptococcus. | Antigens. | | | | | | | | | | | | | | | | | | Homologous antigen. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Left side. | | | | | | | | Right side. | | | | | | | | | | |
| | MB | A84 | A140 | 38D | A102 | B4 | B23 | A141 | A135 | A30 | A179 | R | A65 | A49 | B39 | B29 | B26 | A4 | |
| Ch. | 4 | 3 | 1 | 3 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 3 | 3 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 1 | 1 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 1 | 4 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| B31 | 4 | 4 | 1 | 3 | 4 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 4 |
| | 4 | 4 | 4 | 3 | 4 | 3 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 2 | 1 | 4 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 0 | 3 | 2 | 4 | 2 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 200 | 4 | 4 | 1 | 1 | 4 | 3 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | 4 | 4 | 2 | 2 | 4 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 3 | 4 | 3 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 2 | 3 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 201 | 4 | 4 | 2 | 2 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 3 | 1 | 3 | 3 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 2 | 1 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 3 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| C6 | 4 | 3 | 0 | 1 | 4 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 1 | 4 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 4 | 1 | 2 | 4 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 3 | 2 | 3 | 3 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| 4 | 4 | 3 | 2 | 4 | 4 | 2 | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 0 | 1 | 3 | 3 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 |
| | 2 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| βad | 4 | 1 | 0 | 0 | 4 | 0 | 4 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 1 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 4 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 |
| | 2 | 0 | 0 | 0 | 4 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

The numbers indicate plus signs, and the reading from above downward indicates the following dilutions: the top figure in each group, a dilution of 0.1 cc.; the second figure, 0.05 cc., the third, 0.025 cc.; the fourth, 0.012 cc.

In the tables 4 indicates no hemolysis; 3, 25 per cent hemolysis; 2, 50 per cent hemolysis; 1, 75 per cent hemolysis; 0, negative reaction or complete hemolysis.

two rabbits immunized to non-hemolytic streptococci of the left side and sera of three rabbits immunized to non-hemolytic streptococci of the right side were available. Limited time was responsible for the use of a limited number of sera. The plan was to test the sera immune to hemolytic streptococci against a number of antigens made

TABLE II.

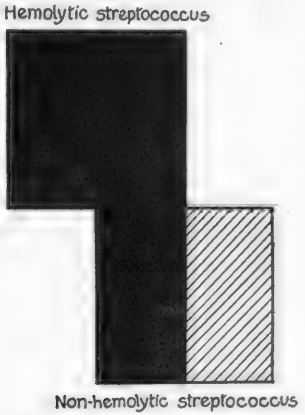
*Relations between Sera Immune to Non-Hemolytic Strains and Antigens of Hemolytic Strains.*

| Sera immune to non-hemolytic strains. | Antigens. | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 4 | B30 | B31 | B31a | 200 | 201 | 202 | 203 | 204 | 205 | 174 | 175 | 182 | 184 | 186 | 189 | 190 | 196 | A96 | C6 | C7 | CK | G | MC | D10 | D14 | βad | β40 |
| Of the | 1 | 4 | 1 | 4 | 3 | 1 | 4 | 1 | 4 | 3 | 1 | 1 | 4 | 1 | 4 | 3 | 4 | 4 | 1 | 1 | 3 | 4 | 1 | 4 | 1 | 4 | 1 | 4 |
| left | 3 | 3 | 1 | 3 | 1 | 1 | 3 | 0 | 3 | 2 | 4 | 1 | 3 | 0 | 3 | 3 | 4 | 3 | 2 | 1 | 1 | 3 | 0 | 3 | 0 | 1 | 0 | 3 |
| side. | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| MB | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A102 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 4 | 4 | 4 |
| | 4 | 4 | 3 | 4 | 3 | 4 | 4 | 2 | 3 | 4 | 4 | 1 | 4 | 3 | 4 | 4 | 2 | 4 | 4 | 3 | 3 | 4 | 3 | 4 | 1 | 3 | 4 | 4 |
| | 2 | 2 | 0 | 1 | 2 | 1 | 2 | 0 | 1 | 0 | 3 | 0 | 3 | 0 | 2 | 3 | 0 | 2 | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 2 | 1 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Of the | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| right | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| side. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B29 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

from non-hemolytic streptococci. Eighteen antigens were used and they are arranged in Table I in the order that they maintained in their previous classification (1). In that classification the first eight antigens were on the left side of the chart, while the remaining ten came in the order printed, on the right side. The methods of im-

munizing animals, of preparing the reagents for the complement fixation reactions, and of performing the tests were the same as described elsewhere (2). Negative and positive controls were used in each test.

The results of these tests are shown in Table I. The lack of reaction with antigens on the right side was very noticeable, while definite reactions only with antigens on the left side was equally striking. The variation in the action of the different sera suggested either variations in the hemolytic streptococci used in producing these sera, or in the relative strength of the sera, or variations in the reactions themselves. The explanation of these variations was not established.




TEXT-FIG. 1. Scheme to represent the relation between hemolytic and non-hemolytic varieties of streptococcus.

Having shown that sera immune to hemolytic streptococci were related in a definitely limited way to non-hemolytic antigens of the left side, we undertook to inquire whether sera immune to non-hemolytic streptococci maintained a similar relation to hemolytic antigens. Table II illustrates the results of these tests. The two sera immune to non-hemolytic streptococci of the left side, gave definite, though at times weak reactions with the entire series of hemolytic antigens. The three sera immune to non-hemolytic streptococci of the right side gave no positive reactions with any of the hemolytic antigens.

In attempting to represent this relation graphically, the non-hemolytic variety can be pictured as a square consisting of two elements as in Text-fig. 1 and the hemolytic variety as a solid homogeneous square. Their relation is indicated by apposing the left side of the non-hemolytic streptococcus to the hemolytic streptococcus.

## DISCUSSION.

The relation between these two varieties of streptococcus as established by these results seems to be orderly and definite. Any classification, to be considered valuable, should be fundamental. This will depend on the criterion used. With streptococci, the older classifications based on morphology and various cultural characteristics have proven unsatisfactory because the criteria do not bear any relation to the more fundamental questions of distribution in man and pathogenicity. These two factors determine the importance of bacteria in medicine. An immunological basis should be the test for classifying pathogenic bacteria.

If analogy with other species is instituted, this classification of the two varieties of streptococci as well as the relation between the two varieties is compatible with certain facts regarding the relative pathogenicity of the two groups. It may be stated that the non-hemolytic streptococcus is a heterogeneous variety, has wide distribution in man, and invades and causes disease only under exceptional conditions of preexisting infection or lowered resistance. The hemolytic streptococci encountered in this study form a unique variety, have very limited occurrence in man, and highly developed invasive and disease-producing qualities.

An analogous situation prevails with pneumococci. Group I pneumococcus is a unique variety (15), is not found in normal throats according to Stillman (16), and has marked pathogenicity. Group IV in the same classification of Dochez is a heterogeneous variety according to Olmstead (17), has moderately widespread distribution in normal throats, but is considered the least pathogenic of the pneumococci. Stillman (16) concludes:

"Among the pneumococci found in the mouths of healthy individuals, Type IV predominates, Type III is fairly frequent, and atypical organisms of Type

II are occasionally encountered. Organisms of these types give rise to a minority of cases of lobar pneumonia."

In the colon-typhoid group it is not possible to institute a perfect comparison because complete immunological studies of the colon group are not available. But the typhoid bacillus has been shown by Hooker (18) to be a nearly homogeneous variety. Its absence from normal individuals and its high invasive and disease-producing powers are well established. By fermentation reactions, at least, the colon bacillus has been shown to be a definitely heterogeneous variety. The other features of occurrence in normal individuals and low pathogenicity are well known.

These considerations indicate the value of the classification of streptococci using the complement fixation reaction as the criterion. They also argue for a fundamental correctness of the classification so established. If it can be proven by further investigation of these points that the variable groups of bacteria are the least invasive while the non-variable groups are the most invasive, a valuable principle in epidemiology would be established.

## CONCLUSIONS.

1. The relation between hemolytic and non-hemolytic streptococci is orderly and arises from the fact that the former variety is unique while the latter is heterogeneous.

2. Analogous considerations of the classification, distribution, and pathogenicity of the streptococcus group and the pneumococcus and colon-typhoid groups show a definite parallelism.

3. These considerations suggest that unique varieties of bacteria associated with man are the more highly invasive, while the heterogeneous varieties are more saprophytic.

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# RENAL FUNCTION IN EXPERIMENTAL HYDRONEPHROSIS.

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PLATES 17 TO 21.

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The object of this investigation is the study of the function of kidneys in the early stages of experimental hydronephrosis by means of the phenolsulfonephthalein test of Rowntree and Geraghty (1). The anatomic changes in hydronephrosis have been thoroughly studied by other observers and will be discussed here only as they pertain to the main purpose of the paper.

## *Anatomic Changes in Hydronephrosis.*

The anatomic changes after complete obstruction of the ureter will be discussed in stages corresponding in duration to the experiments given below.

During the first 3 days only slight changes are noted. Suzuki (2) found a considerable dilatation of the collecting and distal convoluted tubules, but only a slight dilatation of the proximal convoluted tubules at this stage. Fabian (3) noted a slight general dilatation of the tubules as early as 3 hours after ligature of the ureter. In a kidney with hydronephrosis of 3 days' duration I noted a considerable dilatation of the collecting tubules and those portions of Henle's loops lined by flattened epithelium, in both cortex and medulla. The proximal convoluted tubules, however, showed no definite change. The renal pelvis was moderately dilated.

In hydronephrosis of about a week's duration much more pronounced changes develop. Suzuki, in a hydronephrosis of 9 days' duration, found marked dilatation of the capsular spaces and the collecting tubules, and moderate dilatation of the distal convoluted

tubules and both limbs of Henle's loops. The proximal convoluted tubules showed slight or no dilatation. A few casts were found distal to the first convoluted tubule. Casts in the medulla are mentioned by Ponfick (4). Scott (5) found collapse of some tubules in the apical portion of the pyramid, but my observations do not confirm this. In a hydronephrosis of 7 days' duration I observed marked dilation of the ureter and pelvis, and the kidney was distinctly pale. The cortex was thinner in the portions lateral to the sinus than elsewhere. The collecting tubules everywhere are dilated (Fig. 3). The proximal convoluted tubules are unchanged or slightly reduced in size in all parts of the kidney except in the portions lateral to the sinus where they are, for the most part, markedly reduced in size. Numerous casts are found in the collecting tubules in some parts of the kidney.

In hydronephrosis of about 2 weeks' duration the changes have become very pronounced. Suzuki described a narrowing of the parenchyma, most marked in the portions adjacent to the hilus. There was dilatation of the collecting and the distal convoluted tubules. The proximal convoluted tubules and the loops of Henle were usually collapsed, showing beginning atrophy. The atrophy was much more pronounced in the portions adjacent to the hilus than in the mid-sagittal portions. Occasionally a proximal convoluted tubule showed dilatation with a thinned epithelium. Very few casts were noted. In my control animal with a hydronephrosis of 2 weeks' duration the kidney was very pale. The pelvis and ureter were markedly dilated. The parenchyma was thinned, especially in the parts lateral to the sinus. The collecting tubules were all considerably dilated. The proximal convoluted tubules usually showed collapse with a moderate amount of atrophy. Some of these tubules were moderately dilated and had a thin epithelium (Fig. 8). In the portions lateral to the sinus of the kidney the dilatation of the tubules was pronounced and the atrophy of the proximal convoluted tubules was also much greater than in other portions of the kidney. Very few casts were to be seen (Fig. 9).

My observations agree with those of Suzuki that the portions of renal parenchyma lateral to the sinus of the kidney are much more severely injured than other parts of the kidney. The collecting tubules draining these lateral portions of the kidney are entirely col-

lapsed. Suzuki's explanation of this phenomenon seems entirely satisfactory. He points out that the collecting tubules draining these portions of the kidney are subjected to much greater pressure than in other parts of the parenchyma.

In hydronephrosis of about 3 weeks' duration Suzuki noted marked atrophy of the proximal convoluted tubules and dilatation of the collecting tubules. The atrophic changes were much more pronounced in the lateral than in the mesial parts of the renal parenchyma. My observations on a hydronephrosis of 19 days' duration are essentially the same as Suzuki's. In the lateral portions of the kidney the proximal convoluted tubules are in a state of advanced atrophy with relative or absolute increase of the intervening connective tissue. The atrophy of the tubules in the mesial portions of the kidney is not nearly so pronounced. Collecting tubules are everywhere dilated. Only a few casts are to be seen.

Some authors mention areas of lymphocytic infiltration in hydronephrotic kidneys but these were not found in my experimental animals except where tubular atrophy was extreme. Probably most of these lymphocytic infiltrations mentioned by various authors are examples of spontaneous nephritis which is common in rabbits.

*Anatomic Changes in Temporary Hydronephrosis with Subsequent Drainage.*

The investigators mentioned above studied the changes in hydronephrosis without drainage. Others removed the obstruction to the ureter after suitable intervals, established drainage, and studied the subsequent changes.

Rautenberg (6) ligatured the ureter close to the bladder to produce a hydronephrosis. At a second operation an anastomosis was made connecting the dilated ureter and the bladder, and at a third operation the sound kidney was removed. In this manner the changes which were produced in the period of obstruction plus those occurring in the period of drainage could be studied. The duration of hydronephrosis in his experiments varied from 2 to 6 weeks with subsequent drainage even as long as 6 months. He summarized his results as follows: Temporary ligature causes atrophy of the epithelial cells, especially in the convoluted tubules of the cortex. The degree of atrophy varies directly with the duration of obstruction and the injury is not uniformly distributed through the kidney. New connective tissue fills the space left by shrinkage of the tubules.

Some of the atrophic tubules return to normal size with a corresponding decrease of the new connective tissue. The extent of the recovery varies directly with the length of the drainage period. The recovery varies markedly in distribution and degree, sharply contrasted areas of atrophic and normal tubules being found in close association. He believes that the recovery is not permanent. The epithelial cells do not functionate normally, regressive changes again prevail, and eventually complete atrophy of the entire tubule results. The finding of increased blood vessel anastomoses between the kidney and surrounding tissue, claimed by Lindemann (7), was not confirmed by Rautenberg.

Bradford (8) ligatured the ureter on one side in dogs, and after intervals of from 11 to 40 days brought the dilated ureter to the abdominal surface and established a urinary fistula. The animals were kept for 7 to 51 days following the second operation. In three of the experiments a pyonephrosis developed, but the other nine remained free from infection. She found that after the second operation the kidney returned to its normal shape but was reduced to a third or half of its normal size. The ureter remained thickened and dilated. Microscopically there was no general increase of connective tissue, except along the blood vessels. The renal tubules were crowded together, many had disappeared, especially in the cortex, and the epithelial cells were much smaller, with a distinct loss of granulation. These factors accounted for the decrease in the size of the kidney.

The changes in temporary hydronephrosis have been described a so by Corbett (9) and by Amos (10).

### *Functional Studies in Kidneys with Hydronephrosis.*

The studies made of the functional capacity of kidneys with hydronephrosis may be divided into two groups. In the first group hydronephrosis was produced by a complete, sudden blocking of the ureter and functional studies were made at the completion of the desired period of dilatation. In addition, some of the experimenters of this group removed the obstruction, established drainage, and were able to make functional studies during the period of drainage. In the second group hydronephrosis was produced by a constant partial obstruction, sufficient to impede but not to stop the flow of urine.

*Hydronephrosis Produced by Complete Obstruction without Drainage.*—Suzuki studied the function of hydronephrotic kidneys by determining their ability to excrete indigo carmine. The dye was injected shortly before the animals were killed. Judging by the presence of carmine masses in the lumina and carmine granules in the cells of the tubules, he concludes that there is a progressive decrease of function with the duration of the hydronephrosis. In early stages the

function is fairly good, but after 3 weeks it is greatly reduced and is apparently limited to a few convoluted tubules in the medial part of the kidney.

Boetzel (11) produced a unilateral hydronephrosis by ligaturing the ureter close to the bladder, then injected toluidine for functional study, and killed the animals 7 hours following the injection. Toluidine is eliminated by the convoluted tubules. After a dilatation of 6 days' duration no coloring matter was found in the pelvic fluid. After 21 days scarcely any granules, and after 30 days no dye granules were found in the renal cells. If the pressure is released in from 21 to 30 days the kidney excretes some of the dye in the urine, and after some time granules appear in the tubules. From this he concludes that accumulation of dye granules in the cells and excretion are independent, and that in Suzuki's experiments the carmine casts found are due to an abnormal secretion of tubules injured by the carmine itself. He agrees with Rautenberg that kidneys dilated 4 weeks or longer are injured beyond functional power and does not think that all the compressed tubules can recuperate.

Lindemann (12) found an impaired output of indigo carmine from a kidney the ureter of which had been ligatured for only $1\frac{1}{2}$ hours.

Pfaundler (13) obstructed the ureter on one side for periods varying from 15 minutes to 6 hours and analyzed the fluid obtained. He compared the urine from both kidneys and found an increased volume of urine but a decreased concentration of urea and sodium chloride on the obstructed side.

Bainbridge (14) made a unilateral hydronephrosis in cats by ligaturing the ureter. After varying periods of dilatation he measured the pressure of the retained fluid and then analyzed it. After emptying the pelvis he collected the secretion from the two sides simultaneously, using salt as a diuretic. He found a steady diminution in the amount of water and solids excreted by the dilated side, decreasing directly with the duration of the hydronephrosis. The acidity was also diminished. The power of excretion was not entirely lost even when the hydronephrosis had lasted 2 months.

Heidenhain (15) tied one ureter and injected indigo carmine 24 hours later. The animal was killed as soon as the dye appeared in the urine from the normal kidney. The obstructed kidney differed from the normal in showing less color grossly and in the absence of dye granules in the cells of the convoluted tubules. Both kidneys showed carmine in the lumina of the tubules.

Kawasoye (16) ligatured the ureter on one side in nine rabbits and produced hydronephrosis varying from a few hours' to 9 months' duration. 20 minutes before killing the animals he injected indigo carmine. In the shorter periods of dilatation, up to 24 hours, he found a decreased amount of the dye in the tubular epithelium, but after 48 hours no dye whatever was found.

*Hydronephrosis Produced by Complete Obstruction with Subsequent Drainage.*—The studies given above were limited to a determination of the functional capacity of kidneys at the conclusion of a period of dilatation. Other observers drained the dilated kidneys and made subsequent functional observations. Rautenberg, in the series of experiments given previously, drained the dilated kidney into the

bladder after desired periods of dilatation, and at a still later date removed the normal kidney. Seven rabbits with a preliminary hydronephrosis of 42 days and an interval of drainage of from 34 to 155 days died of renal insufficiency a few days after removal of the normal kidney. A similar result was obtained with two rabbits in which the duration of hydronephrosis was 28 and 29 days respectively. However, in a series of three rabbits with hydronephrosis of 3 weeks' duration and a period of drainage of from 22 to 31 days before removal of the normal kidney, two were still living a year later. The third rabbit died of a pyelonephritis. Albumin in small amounts and casts were found in the urine of both the living animals even after a year's time. In a second series of three rabbits with a shorter period of dilatation (about 2 weeks) and nephrectomy after a drainage period of from 14 to 19 days, two died. One was living 39 days after the nephrectomy but it was weak and emaciated. He concludes from these results that there is great individual variation in rabbits. The only functional test which Rautenberg applied was the ability to maintain life and this was lost in kidneys obstructed longer than 3 weeks. That there was still some unhealed renal lesion was shown by the presence of albumin and casts in the urine a year after the removal of the normal kidney.

Kawasoye determined the functional capacity with indigo carmine in a series of seven rabbits in which the obstruction was removed after varying intervals of dilatation. He found a complete restoration of function after a 4 day period and an incomplete restoration after a 7 to 14 day period. No restoration of function whatever, as shown by indigo carmine, was found in kidneys obstructed 21 days.

Corbett tied a ligature around the ureter just tight enough to produce complete obstruction without cutting through the tissues. After desired intervals the abdomen was again opened and the ligature loosened, allowing the urine to flow into the bladder. The normal kidney was removed at the same operation. The functional capacity of the injured kidney was then determined by a study of the total nitrogen and chlorides in the urine. He concludes that kidneys obstructed longer than 10 days do not excrete these substances in normal amounts.

Sollmann, Williams, and Briggs (17) produced a unilateral hydronephrosis of 107 days' duration and then made a urinary fistula for the dilated kidney. During 78 days of drainage no urine was excreted and only two drops were found in the shrunken kidney at autopsy.

*Hydronephrosis Produced by a Partial Obstruction.*—Keith and Snowden (18) tied a rubber band around one ureter to produce partial obstruction. The opposite kidney was removed at the same time. A hydronephrosis resulted in all cases with the fluid under a pressure of from 12 to 30 cm. of water as measured at autopsy. They noted a polyuria, a low specific gravity, and a trace of albumin, in the urine continuously after the operation. The phlorhizin test showed a moderate delay. The blood nitrogen showed a sharp rise at first and then maintained a fairly constant level to within a few days of death, when there was a great increase. The phthalein test was very sensitive and showed a slowing of excretion in the early stages, and in the later stages a progressive reduction in the total excre-

tion when the rise in blood nitrogen occurred. The development of a pyelonephritis is only a question of time in animals operated on by this method.

Hermann (19), Lépine and Porteret (20), and Lindemann determined the effect of a constant back pressure on the kidney secretion by analyzing the urine. In addition to chemical analysis Schwarz (21), Cushny (22), and Filehne and Ruschhaupt (23) noted the effects of diuretics. Similar work on the effect of back pressure on kidney secretion was done by Brodie and Cullis (24) in decerebrated dogs. All are agreed that the flow of urine decreases with high and increases with a low back pressure. With the exception of Brodie and Cullis they found a decreased concentration of solid constituents. For a review of the literature on this phase of the question the reader is referred to Keith and Snowden.

In a recent article Keith and Pulford (25) describe the results obtained in experiments in which bilateral partial obstruction of the ureters was produced by ligaturing with rubber bands. The degree of obstruction obtained was not the same in all the experiments. In five animals definite functional impairment was shown after obstruction lasting about a week and the bands were then removed. Normal function returned in a few days. In another experiment the animal died shortly after removal of the obstruction, but in this case a pyelonephritis complicated the picture. Removal of the bands after 143 days of obstruction, in one experiment, failed to improve the impaired renal function, but observations were continued for only a week.

## EXPERIMENTAL.

### *Method.*

The animals used in the experiments were male rabbits, and all experiments were performed under ether anesthesia. To eliminate all animals in which an already existing renal lesion was present a careful urinalysis and a preliminary phthalein test were made. All rabbits showing any abnormalities in the urine or a phthalein output of less than 65 per cent for 2 hours were discarded. The average phthalein output in the rabbits used was higher than 65 per cent.

The rabbits were operated on in three stages. The preliminary operation consisted in the division and suturing of the ureter in the bladder wall. With all necessary aseptic surgical precautions and under ether anesthesia a median suprapubic incision about 4 cm. long was made exposing the bladder and lower portion of the ureter. The right ureter was then freed at its lower end close to the bladder, care being taken not to injure the blood vessels, ligatured doubly, and sectioned between. An incision about 2 cm. long was

then made in the posterior wall of the bladder through the peritoneal and muscular coats down to the mucosa. The mucosa was separated from the muscular coat for a short distance on both sides and the ligatured ureter was then buried between the mucosa and the muscular coat by suturing the edges of the incision together. The abdomen was then closed.

At the second operation, after varying intervals of time, depending on the duration of hydronephrosis desired, the abdomen was again opened, under ether anesthesia, by an incision close to the first incision and parallel to it. An incision was made through the anterior wall of the bladder. The dilated end of the ureter, buried beneath the mucosa at the previous operation, was clearly visible as an elevation. The mucosa of the bladder over the elevated area was incised and the end of the ureter brought through and opened. The fluid from the dilated ureter then gushed forth into the bladder and drainage was established. The anterior wall of the bladder and abdomen were then sutured.

At the third operation, usually about 1 month after the establishment of drainage, under ether anesthesia, a nephrectomy through a dorsal incision was made on the left side, leaving the hydronephrotic kidney to perform the function alone. The animals were then kept in metabolism cages and the quantity of daily urine was measured. The urine was examined at frequent intervals. A record was kept of the weight. As often as it was deemed necessary phthalein tests were made. When the test returned to normal the animals were killed and the kidneys studied grossly and microscopically. The tissues were fixed in Helly's fluid, embedded in paraffin, and stained with hematoxylin and eosin.

A great many of the experiments were not successful. In a few cases infection from the operation, especially when it was necessary to operate at short intervals, resulted. Sometimes when the abdomen was opened for the second operation it was found that the ligature had slipped off and no hydronephrosis had resulted. Epidemics in the animal house caused the death of many of the experimental animals. In the series reported only those rabbits are included in which it was absolutely certain that the obstruction of the ureter was complete for the length of time indicated.

In order to determine the changes which occurred in the kidneys during the period of drainage, the ureter was ligatured in a number of control rabbits with normal urinary findings. These animals were killed after periods of dilatation corresponding to the duration of the hydronephrosis before drainage in the above experiments. By comparison the extent of recovery during the drainage period was determined.

The phthalein test was made by injecting 1 cc. of the dye into the lumbar muscles. 2 hours later, with aseptic precautions, a No. 10 French, soft rubber catheter was introduced into the bladder. Catheterization is easily performed on male rabbits. The kidney and ureter were then gently pressed to expel as much urine as possible and the bladder was washed out with sterile salt solution. The reading was made in the usual manner in an Autenrieth-Königsberger colorimeter.

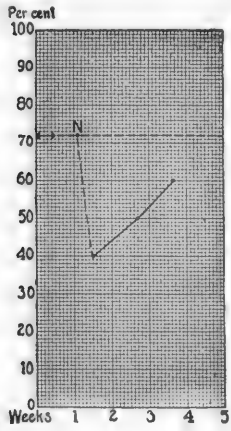
*Experiment 1. Complete Obstruction of the Ureter for 3 Days.*—Male rabbit. Preliminary phthalein 72 per cent. Urine normal. Right ureter ligatured. 3 days later, at the second operation when the ureter was opened, a smoky, brownish fluid gushed into the bladder. 5 days after the second operation the left kidney was removed. The phthalein test was 40 per cent on the 2nd, 50 per cent on the 10th, and 60 per cent on the 17th day after the nephrectomy (Text-fig. 1). A moderate amount of albumin and a few erythrocytes were present for a few days after the nephrectomy, but later the urinary findings were normal. The rabbit died 23 days after the nephrectomy from intestinal obstruction.

The right (hydronephrotic) kidney weighs 6.3 gm. The pelvis and ureter are slightly dilated. Fluid from the pelvis at autopsy shows numerous hyaline and granular casts and one cast of a renal tubule. The lining surface of the pelvis is smooth and shiny. No pus is present. The fluid in the bladder shows a small amount of pus but there is no marked congestion of the bladder wall. The only important difference between the right and left kidneys is the slight dilation of the right pelvis with a corresponding slight thinning of the renal parenchyma anteriorly and posteriorly. Microscopically no important differences are seen.

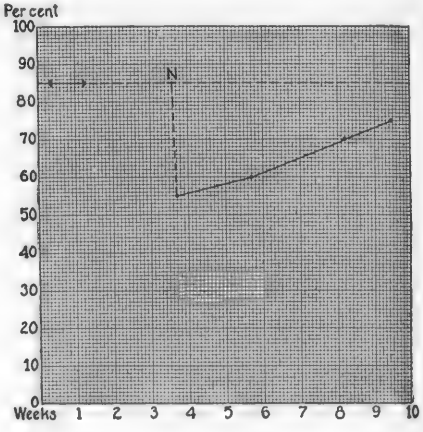
*Experiment 2. Complete Obstruction of the Ureter for 7 Days.*—Male rabbit. Preliminary phthalein test 85 per cent. Urine normal. Right ureter ligatured. 7 days later at the second operation the ureter was found well dilated, about the size of a small goose quill. When it was opened a thin, brownish fluid gushed out under tension into the bladder. Loss of weight between first and second operations 150 gm. 16 days after the second operation left nephrectomy was performed. The phthalein test rose gradually from 55 per cent the day following nephrectomy to 75 per cent 40 days later when the animal was chloroformed (Text-fig. 2). The kidney function had returned to the average normal although not so high as the

original test. The rabbit had increased in weight 280 gm. during the experiment. The urine showed a faint trace of albumin continuously after the nephrectomy but there were no casts, erythrocytes, or pus cells.

The right kidney (hydronephrotic) weighs 6.6 gm. Grossly it shows several linear scars on each side of the pelvis radiating out from the hilus as a center, gradually becoming less distinct and disappearing about two-thirds of the distance to the convex border. They do not cross the rounded convex border. On section these linear scars are seen to correspond to small lateral evaginations of the




TEXT-FIG. 1. Function of a kidney obstructed for 3 days (Experiment 1).




TEXT-FIG. 2. Function of a kidney obstructed for 7 days (Experiment 2).

The ordinates indicate percentage of phthalein, and the abscissæ the time measured in weeks. The horizontal dotted line indicates the level of the preliminary phthalein test. The solid portion of this line indicates the period during which the ureter was tied off. N indicates the point at which the normal kidney was removed, and the phthalein readings are indicated by dots on the charted line.

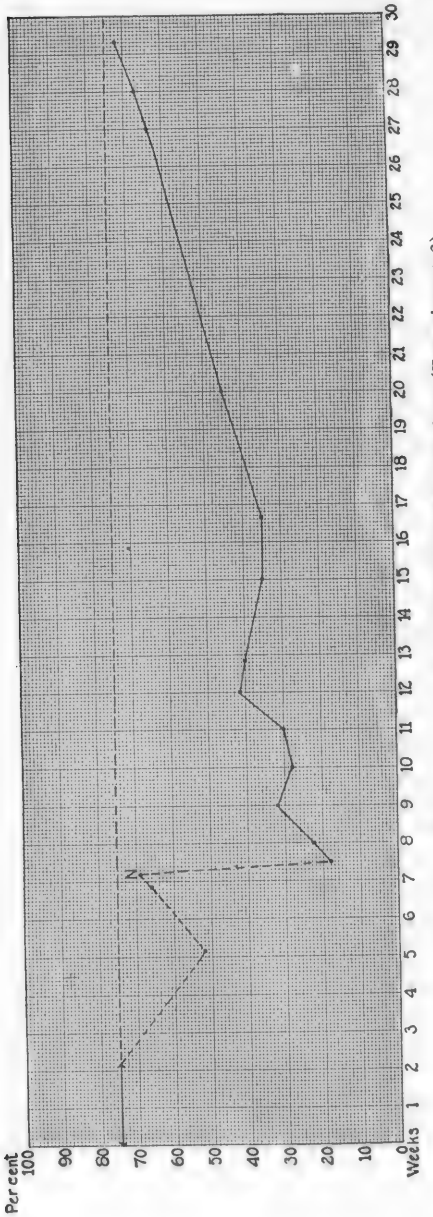
pelvis. The cortical tissue in the linear areas is atrophic, apparently a permanent destruction of the renal tissue due to the hydronephrosis. The cortex shows a uniform thickness of about 4 mm. The microscopic structure of the renal parenchyma is practically normal (Fig. 1) except in the linear scars mentioned above which show atrophy of tubules, small collections of lymphocytes, and increase of connective tissue (Fig. 2). Fig. 3 shows a kidney obstructed by ligature of the ureter for 7 days, and, in all probability, represents the condition of the right kidney of this experiment at the time when drainage was established.

*Experiment 3. Complete Obstruction of the Ureter for 14 Days.*—Male rabbit. Preliminary phthalein test 75 per cent. Urine normal. Right ureter ligatured. 14 days later at the second operation the ureter was found well dilated, about the size of a large goose quill. Brownish fluid gushed out under pressure when it was opened. The phthalein test was 52 per cent 21 days after the second operation, and 66 per cent 30 days after, which was the combined function of the normal and the hydronephrotic kidneys. 35 days after the second operation left nephrectomy was performed. A few erythrocytes, leukocytes, and hyaline and granular casts appeared in the urine for a few days. The phthalein test rose gradually from 18 per cent 2 days after the nephrectomy to 72 per cent 152 days later, when the animal was chloroformed (Text-fig. 3). The phthalein test had returned to within 3 per cent of the original test. The rabbit was active, well nourished, and normal except for a very faint trace of albumin which had been continuously present in the urine since the nephrectomy.

The right kidney (hydronephrotic) weighs 6.8 gm. It presents striking changes grossly. On both the anterior and posterior aspects of the kidney the mesial portion which forms the wall of the sinus is atrophic and depressed, not unlike an old infarct, while the lateral portion along the rounded border is hypertrophied and elevated. The two portions are sharply marked off from each other along a definite line, somewhat irregular at the limits of the pelvis. On section the parenchyma forming the lateral walls of the sinus is very thin and atrophic, apparently a permanent lesion in this region due to the hydronephrosis. The parenchyma along the convex border is hypertrophied, the cortex being 6 mm. in thickness, apparently a compensatory change. The pelvis is dilated and the ureter seems to be the same size as at the second operation 7 months previously.

Microscopic sections show practically normal structure along the convexity of the kidney. Anatomic recovery in this portion is apparently complete (Figs. 4 and 5). A few mitotic figures are seen. There is no increase of connective tissue except in a few small areas. But there is a sudden transition to atrophic tissue where the convex portion joins the flattened lateral walls of the sinus as described in the gross specimen. These lateral portions consist of numerous lymphocytes, markedly atrophic tubules, dilated capsular spaces with atrophic glomeruli, and diffusely distributed fibrous tissue (Figs. 6 and 7). Fig. 8 shows the mesial and Fig. 9 the lateral portion of a kidney obstructed for 14 days, and probably represent the condition of the corresponding portions of the right kidney of Experiment 3 at the time when drainage was established.

*Experiment 4. Complete Obstruction of the Ureter for 17 Days.*—Male rabbit. Preliminary phthalein 70 per cent. Urine normal. Right ureter ligatured. 17 days later at the second operation the ureter was found well dilated, about the size of a large goose quill. A thick brownish fluid gushed out under tension when it was opened. Loss of weight was 200 gm. between the first and second operations. 14 days after the second operation left nephrectomy was performed. The phthalein test was 3.5 per cent on the 3rd day following nephrectomy. The daily




TEXT-FIG. 3. Function of a kidney obstructed for 14 days (Experiment 3).

output of urine increased gradually from 60 cc. on the day following nephrectomy to 250 cc. on the 6th day and remained at about this level. A large amount of albumin, a few erythrocytes, leukocytes, and hyaline and granular casts were constantly present in the urine. The rabbit was very weak and inactive during the entire period and was found dead 9 days after the nephrectomy. The cause of death was apparently renal insufficiency, as no lesions aside from the kidney changes were found at autopsy.

The right kidney (hydronephrotic) weighs 7 gm. There is no evidence of pyelitis or pyelonephritis either grossly or microscopically. The finer structure cannot be studied well because of postmortem autolysis. There are no marked differences between this kidney and one obstructed for the same period without drainage.

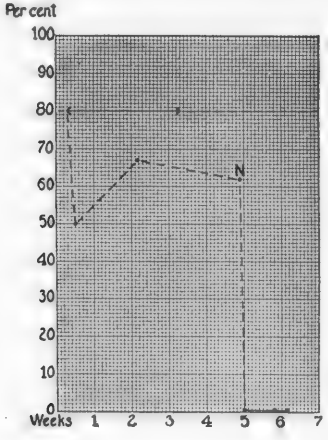
*Experiment 5. Complete Obstruction of the Ureter for 19 Days.*—Male rabbit. Preliminary phthalein 75 per cent. Urine normal. Right ureter ligatured. 19 days later a ureterocystostomy was performed as the preliminary operation was not the usual one but consisted of only ligature and section of the ureter. The ureter was dilated to the size of a large goose quill. A large amount of dark brownish fluid gushed out under tension. Loss of weight of 150 gm. between first and second operations. 18 days after the second operation the rabbit weighed 50 gm. more than at the beginning of the experiment and a left nephrectomy was performed. On the day following nephrectomy the phthalein test was 0. On the 2nd day 20 cc. of urine were excreted showing a tinge of phthalein, a moderate amount of albumin, but no erythrocytes, leukocytes, or casts. The rabbit became very weak and died 3 days after nephrectomy. The urine in the bladder at autopsy showed a minute trace of phthalein. There was no obstruction at the ureteral orifice in the bladder wall. No lesions aside from those in the kidney were found, and death was due apparently to renal insufficiency.

The right kidney (hydronephrotic) weighs 9.1 gm. The pelvis and ureter are markedly dilated. There is no evidence of infection. There are no important changes present, grossly or microscopically, other than those ordinarily found in a simple hydronephrosis of like duration.

*Experiment 6. Complete Obstruction of the Ureter for 19 Days.*—Male rabbit. Preliminary phthalein 70 per cent. Right ureter ligatured. 19 days later at the second operation the ureter was about ¼ inch in diameter and filled with a brownish fluid under tension. 3 days after the second operation left nephrectomy was performed. The phthalein test was 3 per cent on the 2nd day following the nephrectomy. The daily output of urine increased from 40 cc. on the day following nephrectomy to 80 cc. on the 4th day. Urinalysis showed a trace of albumin, a few leukocytes, and a few erythrocytes. The rabbit became very weak and died on the 4th day. At autopsy, besides the renal changes, there was found a small, circumscribed, inspissated abscess in the lower lobe of the right lung. Apparently the cause of death was renal insufficiency, as the lung condition was well walled off and chronic.

The right kidney (hydronephrotic) weighs 12.8 gm. A few subcapsular petechiæ are present. The pelvis and ureter are markedly dilated. There is no evidence of infection. No striking microscopic differences between this kidney and one dilated for a similar period of time without drainage are to be noted.

*Experiment 7. Complete Obstruction of the Ureter for 21 Days.*—Male rabbit. Preliminary phthalein 80 per cent. Urine normal. Right ureter ligatured. The phthalein test was 50 per cent the day following, and 67 per cent on the 13th day after ligature of the ureter. 21 days after the first operation the ureter was found dilated to about ½ inch in diameter and a dark smoky brown fluid gushed out when the opening was made into the bladder. The phthalein was 62 per cent 10 days after the second operation, which was the combined function of the normal and the hydronephrotic kidneys. 11 days after the second operation left




TEXT-FIG. 4. Function of a kidney obstructed for 21 days (Experiment 7).

nephrectomy was performed. The phthalein test was 0 on the day following nephrectomy, but minute traces were present in the urine during the next few days. On the 5th day the phthalein test showed only a faint trace (Text-fig. 4). The amount of urine increased from 2 cc. on the day following the nephrectomy to 100 cc. on the 8th day. A large amount of albumin, with a few erythrocytes, leukocytes, and hyaline casts was continuously present. The rabbit was found dead on the 9th day apparently from renal insufficiency, as no other lesions were present at autopsy.

The right kidney (hydronephrotic) weighs 11.6gm. The pelvis and ureter are markedly dilated. There is no evidence of infection. No marked changes other than those usually found in a simple hydronephrosis of 21 days' duration are present (Fig. 10). A kidney with such extensive changes is unable to function sufficiently to maintain life.

## DISCUSSION.

Hydronephrosis of 3 days' duration causes considerable dilatation of the collecting tubules and the portions of Henle's loop lined by flattened epithelium. No anatomic changes are recognizable in the convoluted tubules. The kidney of Experiment 1 returned to about normal function, as measured by the phthalein test, in 15 days. The only abnormality seen in the kidney after functional recovery is a slight dilatation of the renal pelvis and a slight thinning of the renal parenchyma on the lateral aspects of the renal sinus.

When hydronephrosis has been present for 7 days there is a marked dilatation of the pelvis with considerable thinning of the parenchyma laterally. The dilatation of the collecting tubules is more marked than in the 3 day stage and there is some reduction in size of many of the convoluted tubules, those in the portions lateral to the sinus being especially compressed. The kidney of Experiment 2 had an initial phthalein excretion of 55 per cent and returned to normal function in about 40 days. After functional recovery the kidney showed a dilated pelvis with thinning of the parenchyma laterally. The lateral portions of the kidney showed a few scars due to complete atrophy of small portions of the cortex. Elsewhere the anatomic picture was about normal.

A kidney with hydronephrosis of 14 days' duration shows more dilatation of the pelvis and ureter and greater thinning of the renal parenchyma, especially in the parts lateral to the sinus, than in the 7 day stage. The collecting tubules are considerably dilated and the proximal convoluted tubules are collapsed with some atrophy. The supporting connective tissue is increased. The lateral portions of the kidney show the maximum dilatation of the collecting tubules and maximum atrophy of the proximal convoluted tubules. The initial phthalein output of the kidney in Experiment 3 was 18 per cent, which returned to normal only after 152 days. The pelvis and ureter were still dilated after functional recovery. The portion of renal parenchyma forming the wall of the sinus was completely atrophied. As already stated, Suzuki's explanation of the greater injury sustained by this portion of the kidney seems satisfactory; *i.e.*, the collecting tubules draining this portion, owing to their longer and more indirect course to the papilla are subjected to greater pressure

than those draining the central portion. The destruction of these collecting tubules results in atrophy of their convoluted tubules and glomeruli. The parenchyma along the convex border of the kidney was almost normal, the convoluted tubules having regained their normal size and appearance.

In the kidneys with hydronephrosis longer than 14 days (17, 19, 19, and 21 days) there is marked dilatation of the ureter and pelvis. The proximal convoluted tubules are in a state of advanced atrophy with relative or absolute increase of connective tissue. The collecting tubules are markedly dilated everywhere. No kidney of this group was able to secrete over 3.5 per cent of phthalein. No improvement in secretory power was obtained and no rabbit lived sufficiently long after drainage to develop any notable renal changes other than those seen in simple hydronephrosis of equal duration.

It is to be noted that the ureter and pelvis showed no tendency in any of the experiments to return to normal size. No evidence of secondary infection was seen in the pelvis, ureter, or kidney parenchyma.

If a nephrectomy is performed on a normal animal the remaining kidney excretes, a few days later, nearly as much phthalein as the previous output of both kidneys. The atrophy produced by hydronephrosis greatly reduces this ability until compensatory hypertrophic changes can occur. The kidney with hydronephrosis of 3 days' duration required 15 days to recover 20 per cent of function, or 1.33 per cent per day. The one with hydronephrosis of 7 days' duration required 40 days to recover 20 per cent, or 0.5 per cent per day. The one with hydronephrosis of 14 days' duration required 152 days to recover 54 per cent, or 0.35 per cent per day. It can be readily seen, therefore, that the longer the period of dilatation the slower is the rate of recovery after drainage. The delayed recovery of function in the more prolonged cases of hydronephrosis is apparently due to the greater degree of atrophy of the convoluted tubules. The more advanced the atrophy the slower seems to be the return to normal. The destruction of collecting tubules is not the cause of the atrophy of the convoluted tubules except in the lateral portions of the kidney.

In view of the slow recovery after complete hydronephrosis for 2 weeks it is not surprising that Keith and Pulford noted no appreciable

improvement in a week in a hydronephrosis obtained by partial obstruction of the ureter for 143 days. The rapid recovery noted by them in other experiments after partial obstruction for about a week, when secondary pus infection did not complicate the picture, is also in accordance with the above results.

The contention of Rautenberg that the regeneration after drainage is not permanent and that eventually complete atrophy ensues is not borne out by my experiments. The presence of albumin in the urine in Experiment 3 even at the end of the experiment indicated renal disturbance; but the high phthalein excretion and the autopsy findings showed no evidence of a secondary atrophy.

Rabbits with hydronephrosis longer than 14 days did not live long after removal of the normal kidney. In this group the greatest phthalein output was 3.5 per cent. They all died apparently of renal insufficiency. One may conclude from these results that when a hydronephrotic kidney shows so low a phthalein excretion it will be unable to maintain the life of the individual. The question arises whether such a hydronephrotic kidney would improve sufficiently to maintain life if the normal kidney were not removed until 6 months or more after the establishment of drainage. My observations do not determine this point.

Another point, likewise not determined by the above experiments, is whether the hydronephrotic kidney would recover functionally as it did in Experiment 3 if the normal kidney had not been removed; *i.e.*, whether the stimulus of compulsory function is necessary for recovery.

In the above experiments kidneys obstructed longer than 14 days did not recover; but Rautenberg had two rabbits with hydronephrosis of 3 weeks' duration that lived a year after removal of the normal kidney. This probably means, as Rautenberg suggests, that there is considerable individual variation in rabbits.

## SUMMARY.

Complete obstruction of the ureter causes atrophy of the renal parenchyma, especially marked in the portions lateral to the renal sinus. The longer the duration of the obstruction, the greater is the degree of atrophy.

If the obstruction is removed within 2 weeks the kidney may regain its normal structure except for a varying amount of atrophy in the lateral portions.

Kidneys obstructed for 2 weeks or less may regain their normal function, as measured by the phthalein test.

The longer the period of obstruction, the slower is the rate of recovery. A hydronephrosis of 7 days' duration required 40 days, and one of 14 days' duration required 152 days to recover normal function.

I wish to express my indebtedness to Dr. E. T. Bell for criticisms and suggestions during the progress of this work.

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## EXPLANATION OF PLATES.

### PLATE 17.

FIG. 1. Cortex of the kidney of Experiment 2, showing complete recovery after obstruction of the ureter for 7 days.

FIG. 2. Section through one of the linear scars occurring in the kidney of Experiment 2.

### PLATE 18.

FIG. 3. Cortex of a control kidney with complete obstruction of the ureter for 7 days. Compare with FIG. 1.

FIG. 4. Cortex of the kidney of Experiment 3, from the convex border, showing almost complete recovery after obstruction of the ureter for 14 days.

### PLATE 19.

FIG. 5. High power view of FIG. 4.

FIG. 6. Cortex of the kidney of Experiment 3, from the lateral wall of the sinus of the kidney, showing extreme atrophy after obstruction of the ureter for 14 days.

PLATE 20.

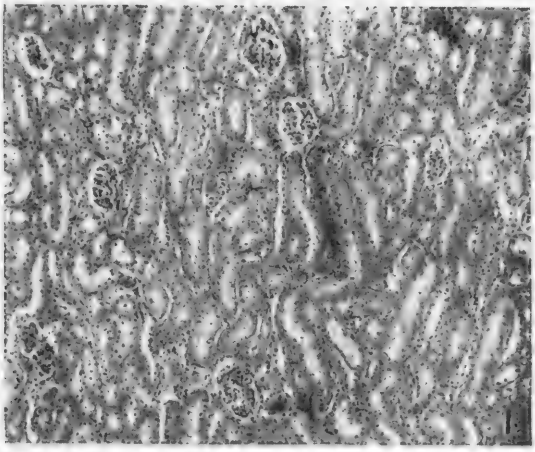
FIG. 7. High power view of Fig. 6.

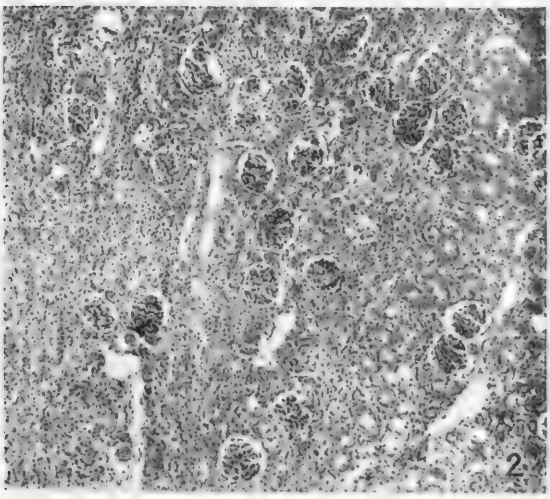
FIG. 8. Cortex from the convex border of a control kidney with complete obstruction of the ureter for 14 days. This portion of the kidney recovers after drainage. Compare with FIG. 4.

PLATE 21.

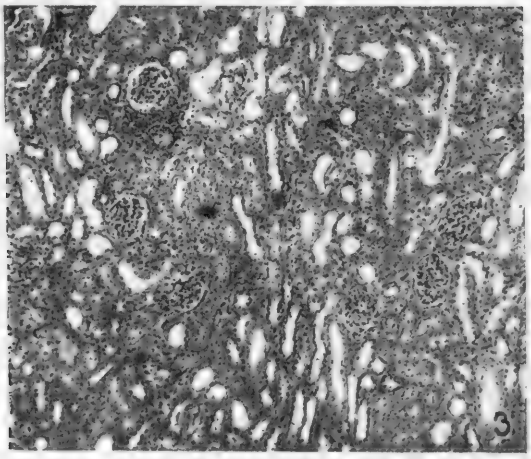
FIG. 9. Cortex from the lateral wall of the sinus of a control kidney with complete obstruction of the ureter for 14 days. This portion of the kidney does not recover after drainage. Compare with FIG. 6.

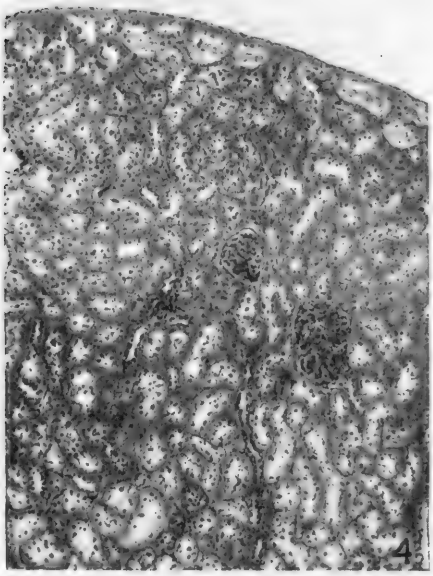
FIG. 10. Cortex of the kidney from Experiment 7 with complete obstruction of the ureter for 21 days.



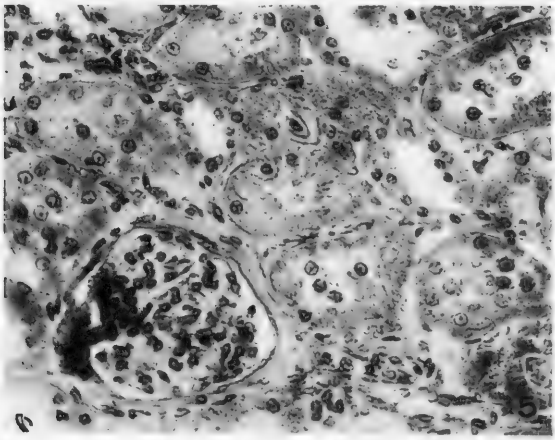


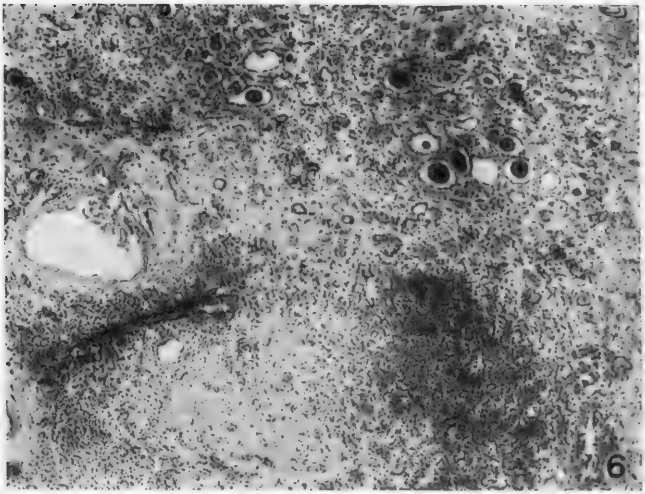

(Johnson: Renal function in hydronephrosis.)



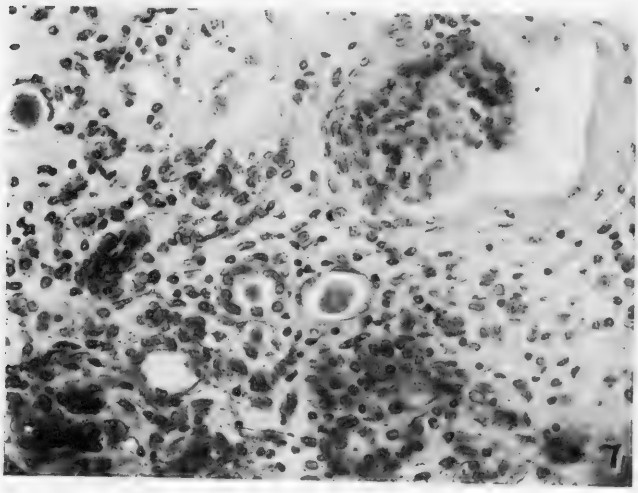



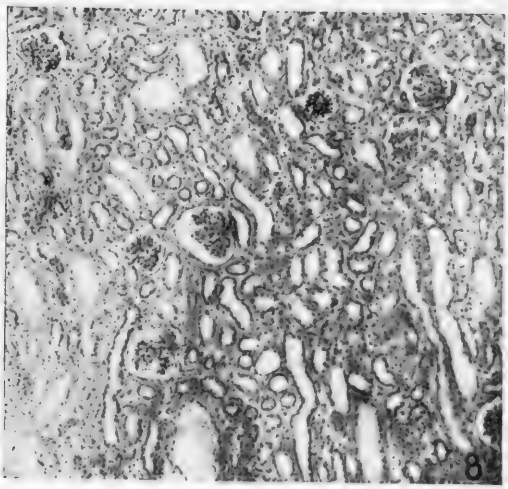

(Johnson: Renal function in hydronephrosis.)



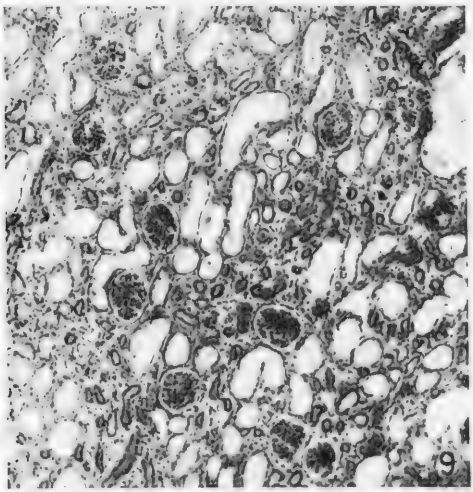


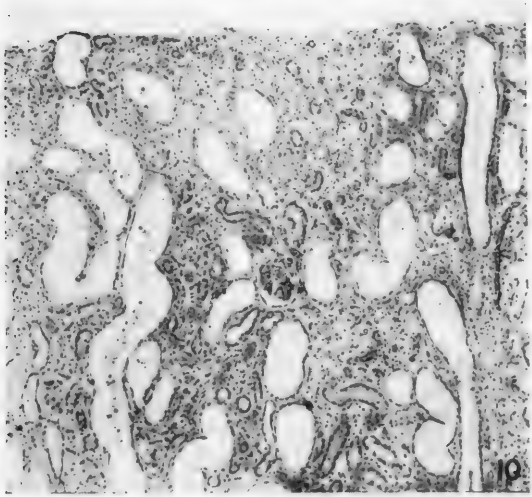
(Johnson: Renal function in hydronephrosis.)





(Johnson: Renal function in hydronephrosis.)





(Johnson: Renal function in hydronephrosis.)

# PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

## III. Toxic Protein Catabolism and Its Influence upon the Non-Protein Nitrogen Partition of the Blood.

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(Received for publication, March 15, 1918.)

This paper contains experimental data which supplement the published experiments of Whipple, Cooke, and Stearns (1, 2) who showed the profound influence of proteose injections upon the elimination of nitrogen in the urine. A single injection of toxic proteose in a fasting dog will cause a great rise in the base-line level of nitrogen elimination —often an increase of 4 to 6 gm. above normal, lasting many days. This, of course, indicates a great destruction of body protein following a single intravenous toxic injection—an acceleration of tissue autolysis lasting over 2 to 7 days with a maximum nitrogen elimination occurring during the second 24 hour period. The toxic proteose causes a disturbance of tissue equilibrium which is not restored to normal for several days following a severe intoxication. It has been suggested that the toxic proteose may so injure cell protoplasm that the resultant cell autolysis may form other toxic split products capable of further injury to the body—a true vicious circle of intoxication (1). For this reason it seemed desirable to study the blood by all available methods to determine the nitrogenous products of increased tissue autolysis *in vivo*.

The term "proteose intoxication" is used in a liberal sense in this communication. It is generally admitted that the chemistry of the proteoses is at best unsatisfactory. There are few proteoses which can be accepted as chemically pure by a critical chemist and very few indeed that will meet the requirements for pure proteoses demanded

by Gibson (3). It must be admitted that even the pure proteos preparations may be mixtures of several proteoses. It is claimed that these pure proteoses are relatively inert, but we believe it is safer to say that the complicated methods used to purify the proteoses will completely denature these several proteoses. Repeated precipitation of the proteoses used in our experiments by means of alcohol will completely remove all toxicity, yet this does not mean a removal of toxic impurities, because a collection of all the alcoholic filtrates will likewise show no toxic substance. The whole question of isolation of pure proteoses is much like that of the isolation of pure ferments. The process of isolation destroys the toxicity of the proteose and the activity of the ferment.

The "proteose solutions" employed in our experiments are prepared from the material obtained from human or animal intestinal obstruction or closed intestinal loops by means of alcoholic precipitation, solution of the precipitate in water, and removal of albumin by heat in a dilute acid solution (1). A second alcoholic precipitation may be used but this will destroy some of the original toxicity. The fluid is slightly opalescent or clear broth-like with a faint amber color. In the concentration used, the solution usually contains about 5 mg. of dried substance per cubic centimeter. We hope to report in the near future on a chemical study of this material. We realize that this preparation may contain one or more primary proteoses and perhaps some $\beta$-nucleoprotein and nucleohistone.

The important fact remains that the proteose solution contains a substance or substances which are not present in the normal intestine but are abundant in the obstructed intestine (human or animal). It is highly probable at least that this "proteose" is concerned in the intoxication of intestinal obstruction which is perhaps as typical an example as may be found of true non-specific intoxication. All infections have an important non-specific intoxication factor which may not be very unlike this non-specific intoxication of intestinal obstruction. We believe that information concerning the non-specific intoxication of intestinal obstruction will be of value for a proper comprehension of the non-specific fraction of the general intoxication present in bacterial infections.

### *Methods.*

Urea was determined as described by Van Slyke and Cullen (4).

The amino nitrogen, peptide nitrogen, and total non-protein nitrogen were determined as follows:[1] 15 cc. of blood were treated for 15 to 30 minutes with 1 cc. of a 10 per cent solution of Squibb's urease. The proteins were then precipitated by diluting to 150 cc. with 2.5 per cent trichloroacetic acid. The filtrate was received in a measuring cylinder and the volume noted. When the drainage had practically stopped, the filtrate was transferred to a beaker and boiled 15 minutes to decompose the trichloroacetic acid. A few drops of saturated potassium carbonate solution were added to render the solution alkaline to phenolphthalein. The solution was then concentrated at 20–30 mm. pressure to remove ammonia and reduce the volume to a few cubic centimeters. It was finally transferred from the distilling flask to a 15 cc. measuring flask.

1 cc. duplicates were used for determination of total nitrogen by the micro-Kjeldahl technique of Folin and Farmer (5), the ammonia being titrated with 0.02 N acid and alkali. 2 cc. portions of the solution were used for amino nitrogen determination (6). For peptide nitrogen a 5 cc. portion was mixed with 5 cc. of concentrated hydrochloric acid and heated 24 hours at 100°C. to hydrolyze peptides. The greater part of the free hydrochloric acid was removed by concentration nearly to dryness under diminished pressure in a 50 cc. distilling flask. The residue was taken up with about 20 cc. of water, rendered alkaline to phenolphthalein with a few drops of saturated potassium carbonate solution, and concentrated again nearly to dryness to remove ammonia. The residue was brought to 5 cc. volume and 2 cc. portions were used for amino nitrogen determination, the value determined being that of the amino nitrogen plus the peptide nitrogen, which is converted into amino nitrogen by the acid hydrolysis.

## EXPERIMENTAL OBSERVATIONS.

*Dog 18–12* (*Table I*).—Mongrel, adult male. This dog was injected intravenously under ether anesthesia with a proteose solution prepared from material obtained from closed loops of the small intestine of the dog. A large dose, 260 cc., was given slowly and was associated with considerable fall in blood pressure. Vomiting and diarrhea appeared during the 1st hour after injection. During the 2nd hour the diarrhea became blood-tinged. Respiration was slow and deep; vomiting at intervals; pulse weak. After this the condition of the dog showed little change. The temperature showed slight rise with a fall shortly before death. 5.25 hours after injection animal was moribund; given ether and killed.

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[1] The methods will be discussed in more detail in *The Journal of Biological Chemistry*. The present outline is, however, sufficient to permit repetition of the experiments.

*Autopsy.*—Typical of acute proteose intoxication described previously (7). Blood clots slowly. The spleen and liver are intensely engorged and deep purple in color. The duodenum, jejunum, and ileum show intense engorgement of the mucosa, which is velvety and deep purplish red. There is much fluid in the intestine.

TABLE I.

*Dog 18–12. Acute Intoxication. Proteose Injection.*

| Time after injection. | Total non-protein nitrogen. | Non-protein nitrogen per 100 cc. of blood as. | | | | Remarks. |
|---|---|---|---|---|---|---|
| | | Urea. | Non-urea. | Amino nitrogen. | Amino nitrogen plus peptide nitrogen. | |
| *hrs.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | |
| Before. | 45.5 | 10.8 | 34.7 | 11.0 | 18.8 | Weight 26.3 lbs. |
| After. | 47.9 | 11.5 | 36.4 | 12.2 | 22.4 | Immediately after injection. |
| 2.25 | 53.5 | 15.7 | 37.8 | 12.3 | — | |
| 3.75 | 62.6 | 20.7 | 41.9 | 13.2 | 25.8 | |
| 5.25 | 61.8 | 22.9 | 38.9 | 13.7 | 24.6 | Autopsy typical. |

*Dog 18–19* (*Table II*).—Fox-terrier, adult female. Under ether anesthesia the proteose solution was given intravenously, 110 cc. in amount. The proteose was prepared from a human case of intestinal obstruction, material being removed at operation. Injection caused a moderate fall in blood pressure. Vomiting began within 30 minutes. After 1 hour a little semifluid feces was passed. The temperature became subnormal; pulse very weak. 5 hours after injection dog is prostrated; pulse barely palpable. 6 hours after injection dog in severe shock but would live perhaps 1 or 2 hours longer. Ether anesthesia; killed.

*Autopsy.*—Performed at once; picture identical with that described for Dog 18–12. Lesions are typical of an acute proteose intoxication.

*Dog 18–25* (*Table II*).—Mongrel, young adult male. Under ether anesthesia a proteose solution, 175 cc., was injected intravenously. This had no effect upon the blood pressure. Vomiting began in 30 minutes and the temperature, which had risen slightly, fell gradually until death. The clinical picture is similar to that of the dogs described above. After 3.5 hours dog moribund and was killed.

*Autopsy.*—Performed at once; picture typical of proteose intoxication, as described for Dog 18–12.

*Dog 18–28* (*Table II*).—Mongrel, young adult male. Under ether anesthesia a proteose solution, 150 cc., was injected intravenously. This proteose was obtained from a case of human intestinal obstruction. There was considerable fall of blood pressure during and after the injection. A progressive fall in temperature developed during the course of the intoxication. Vomiting began 20 minutes

after injection and diarrhea within an hour. The clinical picture is identicalwith that described above. After 4.1 hours the dog is in severe shock but might live 1 hour longer. Ether anesthesia; killed.

*Autopsy.*—Performed at once; findings are typical of acute proteose intoxication, as described for Dog 18–12.

TABLE II.

*Acute Intoxication. Proteose Injection.*

| Time after injection. | Total non-protein nitrogen. | Non-protein nitrogen per 100 cc. of blood as. | | | | Remarks. |
|---|---|---|---|---|---|---|
| | | Urea. | Non-urea. | Amino nitrogen. | Amino nitrogen plus peptide nitrogen. | |
| | | | Dog 18–19. | | | |
| *hrs.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | |
| Before. | 33.0 | 9.0 | 24.0 | 9.9 | 16.4 | Weight 13.4 lbs. |
| After. | 37.1 | 10.1 | 27.0 | 10.8 | 18.7 | |
| 6.0 | 49.9 | 22.9 | 27.0 | 10.8 | 18.7 | Killed. |
| | | | Dog 18–25. | | | |
| Before. | — | — | — | — | — | Weight 22.5 lbs. |
| 3.5 | 37.2 | 11.5 | 25.7 | 11.1 | 20.1 | Killed. |
| | | | Dog 18–28. | | | |
| Before. | — | — | — | — | — | Weight 17.3 lbs. |
| 4.1 | 59.5 | 19.6 | 39.9 | 12.1 | 20.3 | Killed. |

*Dog 18–21 (Table III).*—Mongrel collie, adult female. Ether anesthesia. Laparotomy with section of small intestine 12 inches below the duodenojejunal junction. The sectioned ends turned in and united to produce complete obstruction. During the first 2 days after the operation the dog showed no clinical evidence of intoxication; no vomiting. On 4th day vomiting of bile-stained fluid began. There was slight fall in temperature. 5th day, vomiting continued. Dog is quite sick. 5 p.m. Ether anesthesia; killed.

*Autopsy.*—Performed at once. The peritoneal cavity contains 350 cc. of pale, thin, yellow, turbid fluid. Peritoneal surfaces show acute inflammation. A peritonitis resulted from necrosis of the intestine at site of obstruction with a slight escape of intestinal contents. The peritonitis is probably of short duration. Abdominal viscera negative except for cloudy swelling. Small intestine above obstruction shows definite engorgement of mucous membrane. There is little fluid in the intestine. Blood collected in dry oxalate shows little if any blood concentration.

*Dog 18–50* (*Table III*).—Small bulldog, male; weight 12 pounds. Ether anesthesia, laparotomy, and production of complete obstruction in the middle of the small intestine. Obstruction produced by section of intestine and inversion of cut ends. Vomiting began on the 2nd day after operation. There is little intoxication until the 6th day; on the 7th day after operation dog is intoxicated, vomiting gray, foul smelling fluid. Pulse is weak. Ether anesthesia; killed.

*Autopsy.*—Performed at once. Wound is clean. Peritoneal surfaces smooth and moist. The small intestine above the obstruction is dilated and thickened.

TABLE III.

*Intoxication of Intestinal Obstruction.*

| Time after operation. | Total non-protein nitrogen. | Non-protein nitrogen per 100 cc. of blood as. | | | | Remarks. |
|---|---|---|---|---|---|---|
| | | Urea. | Non-urea. | Amino nitrogen. | Amino nitrogen plus peptide nitrogen. | |
| | | | Dog 18–21. | | | |
| *days* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | |
| 2 | — | 7.9 | — | — | — | Obstruction in midjejunum. |
| 3 | — | 6.5 | — | — | — | |
| 5 | — | 55.2 | — | — | — | 10.30 a.m. |
| 5 | 79.5 | 52.8 | 26.7 | 10.8 | 22.6 | 5.10 p.m. Killed. |
| | | | Dog 18–50. | | | |
| 7 | 137.2 | 97.9 | 39.3 | 9.6 | 17.0 | Obstruction in low jejunum. |
| | | | Dog 18–41. | | | |
| 2 | 87.1 | 55.0 | 32.1 | 12.7 | 16.8 | Obstruction in low jejunum. |
| | | | Dog 17–222. | | | |
| 25 | 39.2 | 16.3 | 22.9 | 4.3 | 7.9 | Intestinal loop of ileum. |

Other organs negative. The jejunum contained the usual amount of creamy-grayish, semifluid material which contains large amounts of toxic proteose.

*Dog 18–41* (*Table III*).—Fox-terrier, adult male; weight 22 pounds. October 4, 1917. Ether anesthesia and extirpation of head of pancreas. Both arms of the pancreas isolated and left intact in the peritoneum. Dog recovered perfectly following this operation. November 6. Dog in good condition; weight 19 pounds. Ether anesthesia and simple obstruction in middle of small intestine. Day following operation animal is sick. Temperature normal. 48 hours after operation dog is severely shocked with subnormal temperature; would probably die within a few hours. Ether anesthesia; killed.

*Autopsy.*—Performed at once. Peritoneal cavity clear. The pancreas shows a good deal of induration and atrophy but the parenchyma is present in considerable amounts. The obstructed intestine contains about 450 cc. of creamy, brown, thick fluid.

*Dog 17–222* (*Table III*).—Mongrel collie, female; weight 20.5 pounds. Ether anesthesia with isolation of a closed loop of the ileum beginning just above the ileocecal valve measuring 100 cm. in length. Ileum united around the loop by enteroenterostomy. Dog recovered well after operation. During the 2nd week occasional attacks of vomiting. There was evidence of chronic intoxication with gradual loss in weight. 25 days after operation weight was 17 pounds. Dog is not acutely intoxicated. Ether anesthesia; killed.

*Autopsy.*—Performed at once. Peritoneum is clean. The isolated loop contains 900 cc. of thick, creamy, brown fluid. The intestinal walls are hypertrophied and thickened. There is no ulceration of the mucous membrane. The rest of the gastrointestinal tract is negative. Other findings have no significance.

TABLE IV.

*Normal Dog after Meat Feeding.*

| Time after meat feeding. | Total non-protein nitrogen. | Non-protein nitrogen per 100 cc. of blood as. | | | | Remarks. |
|---|---|---|---|---|---|---|
| | | Urea. | Non-urea | Amino nitrogen. | Amino nitrogen plus peptide nitrogen. | |
| *hrs.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | |
| 0 | 29.7 | 9.8 | 19.9 | 10.1 | 15.8 | Weight 14.1 kg. Fed 290 gm. of cooked ground beef heart. |
| 0.5 | 30.0 | 10.3 | 19.7 | 10.1 | 14.5 | |
| 1.5 | 38.3 | 15.3 | 23.0 | 10.7 | 15.5 | |
| 3.0 | 46.9 | 22.4 | 24.5 | 9.9 | 14.8 | |
| 5.0 | 51.7 | 28.4 | 23.3 | 9.6 | 15.1 | |
| 8.0 | 50.7 | 29.5 | 21.2 | 10.4 | 15.9 | |

DISCUSSION.

The results show that intoxication by injected proteose causes an immediate and rapid increase in autolysis of body protein. The effect is so marked that the increase in blood urea is comparable with that accompanying the digestion of a heavy feeding of meat. The other non-protein nitrogenous constituents are slightly increased, due almost entirely to increases in free amino acids ($NH_2$) and peptides (NH). The entire picture of the non-protein blood nitrogen is indistinguishable from that following a heavy protein meal (Table IV).

The same remarks apply to the intoxication following intestinal obstruction. The urea is enormously increased over the usual fasting value, indicating a protein catabolism so rapid that the kidneys fail to keep pace with it. The non-protein nitrogenous products of the blood other than urea are not appreciably altered.

There is no evidence to indicate that the intoxication results from the tissue autolysis. The reverse appears to be the case, because in no instance does the urea concentration reach a toxic level, while the other nitrogenous products are not increased at all beyond usual limits. Also, in Dog 18–25, Table II, death occurred before autolysis had gone far enough to raise even the urea beyond that observed in fasting.

On the other hand, the results present a good example of accelerated protein catabolism and tissue autolysis caused by the action of a toxin, uncomplicated by the presence of parasites, by abnormally high temperature, or by any other apparent factors save the toxin itself.

The fact that the peptide nitrogen of the blood is not increased to an abnormal degree by the intoxication does not exclude the possibility that among the products of the induced autolysis there may be toxic proteoses which add their effect to that of the injected or absorbed proteose. The amounts of such proteoses required to intoxicate are too little to increase measurably the peptide nitrogen of the blood.

## SUMMARY.

The acute intoxication following an injection of a toxic proteose is usually associated with a large increase (40 per cent or more) in the non-protein nitrogen of the blood. This increase is found chiefly in the blood urea nitrogen, but the amino and peptide nitrogens also may show small increases. The changes observed in the blood non-protein nitrogen are identical with those which follow the feeding of large amounts of meat (8).

These facts indicate that the proteose intoxication causes an abnormally rapid autodigestion of tissue proteins, but that the nitrogenous end-products are, in chief part at least, the same that result from normal catabolism of food proteins. There is no evidence that the

autolytic products play any part in causing the intoxication. The possibility of such a part and a resultant vicious circle is not excluded, but from the available facts the autolysis appears more as a result rather than cause of the intoxication.

It appears possible that in disease or intoxication tissue catabolism may be enormously accelerated and yet yield the end-products of normal protein metabolism.

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# PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

## IV. The Metabolism of Dogs with Sterile Abscess, Pancreatitis, and Pleuritis.

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(Received for publication, March 15, 1918.)

We wish to present these experiments as a contribution to the study of sterile inflammatory processes—true non-specific inflammation. There can be no question of bacterial toxins, hypothetical endotoxins, or specific reactions. The injury is a chemical injury of tissue and body wandering cells. The reaction on the part of the body must be due to products absorbed from the areas of injury and inflammation. The substances responsible for the general reaction are almost certainly formed from body protein by autolysis and autolytic ferments are conspicuously present. Earlier investigators have been able to isolate proteoses, peptones, and a variety of other protein split products from certain inflammatory exudates. Proteoses have been isolated recently from various normal tissues and organs by Abel, Pincoffs, and Rouiller (1). We have been able to isolate from certain inflammatory exudates proteose-like substances which are toxic and hope to report on this work in the near future. Whatever these substances may be, formed in inflammatory sterile exudates, they are rapidly absorbed into the general circulation causing a variety of reactions, leukocytosis, fever, loss of weight, etc. The rise in nitrogen output is very striking and reaches as high a level in a fasting dog with a sterile abscess as with a staphylococcus abscess. From the clinical signs and from the nitrogen metabolism no difference can be detected between an abscess due to turpentine and an abscess due to a staphylococcus. We must conclude that the greater part of this

reaction due to the staphylococcus is non-specific. We shall emphasize constantly the important non-specific fraction of all intoxications due to specific bacterial agents.

### *Methods.*

The experiments were all carried out on fasting dogs, which were kept in standard metabolism cages and allowed to reach a relatively constant level of nitrogen elimination before any experiment was performed. 24 hour collections of urine were made and nitrogen was determined in duplicate by the Kjeldahl method. Details of the methods for collection of urine and feces and for non-protein nitrogen and urea in the blood have been described elsewhere (2,3). All operative procedures were done under morphine and ether anesthesia. When irritant material was injected, the animal was given morphine.

### EXPERIMENTAL OBSERVATIONS.

*Dog 16–46* (*Table I*).—Short haired fox-terrier, male; weight 27 pounds. The dog was allowed to fast 6 days and placed in a metabolism cage. 2 days later 1.25 cc. of turpentine were injected subcutaneously. The clinical signs of intoxication which followed during the formation of the abscess disappeared when the abscess was opened. Abscess contents sterile. Recovery was uninterrupted and the wound healed well.

*Dog 17–9* (*Table II*).—Large strong black-and-tan, adult male; weight 35.5 pounds. The dog was allowed to fast 9 days and placed in a metabolism cage. 2 days later 1.5 cc. of turpentine were injected subcutaneously over the shoulder. A large abscess of about 10 cm. diameter developed, and was opened 3 days later, 120 cc. of thick creamy pus being removed. This was sterile. 2 days later a slight nasal discharge developed and later a cough. These symptoms continued with varying intensity for 13 days, when the experiment was discontinued. At this time the animal was dull and listless but showed no frank signs of any localized lesion. The dog recovered promptly when fed. The abscess sinus, meanwhile, drained, granulated, and was almost healed when observations were discontinued.

*Dog 16–176* (*Table III*).—Strong bulldog, young male; weight 25.5 pounds. The dog was allowed to fast 5 days and placed in a metabolism cage. 3 days later 1 cc. of turpentine with 2 loopfuls of a 24 hour agar culture of *Staphylococcus aureus* was injected subcutaneously over the thorax (morphine $\frac{1}{8}$ grain). A typical abscess developed with fever and moderate intoxication. The abscess ruptured in about 60 hours and the healing was uninterrupted.

The preceding group of experiments (Tables I, II, and III) are illustrative of a considerable number of experimental observations

which need not all be tabulated because the reaction is so uniform. A turpentine abscess causes a remarkable rise in urinary nitrogen, often 100 to 200 per cent daily increase following the development of the abscess. Still more interesting is the great increase of urinary

TABLE I.

*Dog 16–46. Sterile Abscess (Turpentine).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | *°C.* | *lbs.* | |
| 1 | 2.55 | 225 | — | 24.9 | Fasting 6 days. |
| 2 | 2.59 | 152 | — | 24.5 | |
| 2 | Turpentine, 1.25 cc., injected intravenously. | | | | |
| 3 | 2.69* | — | 40.3 | 24.1 | Clinically sick. |
| 4 | 5.88 | 458 | 39.8 | 24.0 | Abscess forming. |
| 5 | 5.85 | 509 | 40.1 | 23.5 | " soft. |
| 6 | 5.82 | 825 | 39.9 | 23.1 | |
| 6 | 1 p. m. Abscess opened; 150 cc. of pus containing 1.04 gm. of nitrogen. | | | | |
| 7 | 7.69 | 740 | 38.3 | 22.1 | Wound granulating. |
| 8 | 3.84 | 251 | 37.9 | 21.6 | Clinically well. Feces nitrogen 0.97 gm. |
| 9 | 3.32 | 340 | 38.0 | 21.1 | |
| 10 | 2.86 | 125 | 37.7 | 20.8 | Wound clean. |
| 11 | 3.22 | 130 | 37.9 | 20.4 | |
| 12 | 2.88 | 180 | — | 20.0 | |
| 13 | 2.97 | 115 | 38.1 | 19.6 | |
| 14 | 2.74 | 105 | — | 19.5 | |
| 15 | 2.72 | 81 | — | 19.0 | |
| 16 | 2.52 | 50 | — | 18.9 | Wound 1.5 cm. in diameter and healing. |

* Feces and cage washings included.

nitrogen on the day following the rupture or drainage of the abscess, at a time when the clinical picture is normal. We must assume that this represents an escape of nitrogenous material which has been held somewhere in the body (blood and tissues) during the period of intoxication. The amount found in excess in the blood the day be-

fore the abscess rupture or drainage cannot account for all of this excess output on the day following operation and drainage.

An infected abscess with or without a chemical irritant gives the same increase in urinary nitrogen excretion but has a tendency to

TABLE II.

*Dog 17–9. Sterile Abscess (Turpentine) Complicated by Canine Distemper.*

| Day. | Urine. Nitrogen. | Urine. Amount. | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | *gm.* | *cc.* | *°C.* | *lbs.* | |
| 1 | 3.25 | 100 | 38.1 | 31.9 | Fasting 9 days. |
| 2 | 3.14 | 107 | 38.0 | 31.4 | |
| 2 | Turpentine, 1.5 cc., injected subcutaneously. | | | | |
| 3 | 6.41* | 235 | 39.0 | 31.3 | Drinks much water. |
| 4 | 9.44 | 454 | 39.2 | 31.1 | |
| 5 | 11.48 | 702 | 38.7 | 30.2 | |
| 5 | Abscess opened; pus contained 0.756 gm. of nitrogen. | | | | |
| 6 | 11.91 | 720 | 38.4 | 28.6 | Feces nitrogen 1.37 gm. |
| 7 | 6.96 | 260 | 38.2 | 27.8 | Nasal discharge. |
| 8 | 5.99 | 382 | 38.1 | 27.4 | " " |
| 9 | 5.68 | 196 | 38.1 | 27.1 | " " |
| 10 | 5.99 | 188 | 37.9 | 26.6 | " " coughing, and sneezing. |
| 11 | 5.56 | 178 | 38.0 | 26.0 | " " no cough. |
| 12 | 6.34 | 206 | 37.6 | 25.5 | " " " " |
| 13 | 6.42 | 200 | 38.1 | 24.8 | " " " " |
| 14 | 6.73 | 205 | 37.1 | 24.6 | " " " " |
| 15 | 7.36 | 210 | 37.1 | 24.2 | No nasal discharge. |
| 16 | 7.54 | 245 | 36.8 | 23.6 | " " " |
| 17 | 8.89 | 262 | 36.6 | 22.9 | Definite nasal discharge; no cough. |
| 18 | 6.45 | 257 | 36.8 | 22.4 | " " " sick and dull. |
| 19 | 10.64 | 355 | 36.4 | 22.0 | " " " " " " |
| | | | | | Discontinued. |

* Slight amount of feces included.

spontaneous rupture which is not so pronounced in the sterile abscess. Distemper is a troublesome infection of dogs probably due to *Bacillus bronchisepticus*, which usually localizes in the nasal, pharyn

geal, and bronchial mucous membranes. This infection will invariably cause a considerable rise in the urinary nitrogen output of a fasting dog, and this possibility must be kept in mind in all experiments of this nature with dogs. It is practically certain that this dog (Table II) developed the disease during the experiment as the control period of nitrogen elimination is normal. This complication does not obscure the abscess reaction.

TABLE III.

*Dog 16–176. Bacterial Abscess (Staphylococcus and Turpentine).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | *°C.* | *lbs.* | |
| 1 | 3.16 | 65 | 38.0 | 22.6 | Fasting previous 5 days. |
| 2 | 2.72 | 85 | 38.0 | 22.2 | |
| 3 | 2.86 | 62 | 38.0 | 21.6 | |
| 3 | Injection of 1 cc. of turpentine and 2 loopfuls of *Staphylococcus aureus* subcutaneously. | | | | |
| 4 | 4.24 | 139 | 39.3 | 21.6 | Abscess size of fist. |
| 5 | 4.96 | 138 | 39.6 | 20.9 | " soft. |
| 6 | 6.29 | — | — | 20.3 | " ruptured. |
| 7 | 5.15 | 177 | 38.4 | 19.6 | |
| 8 | 3.28 | 95 | 38.0 | 19.1 | Feces nitrogen 0.21 gm. |
| 9 | 2.98 | 80 | 38.2 | 18.6 | |
| 10 | 3.05 | 65 | 38.2 | 18.2 | Discontinued. Wound doing well. |

Diuresis is a noticeable feature in these experiments. The dogs were allowed to drink water as they wished and not given any fixed amount by stomach tube.

*Dog 16–46 (Table IV).*—Strong fox-terrier. The dog was allowed to fast 5 days and placed in a metabolism cage. Operation was done under morphine-ether anesthesia. Sterile bile was injected into the pancreatic duct and the duct then tied; abdominal wound closed as usual. 2 months later the pancreas was examined and showed only few adhesions and puckerings on the gland surface.

*Dog 16–172 (Table V).*—Young black long haired mongrel; weight 15.2 pounds. The dog was allowed to fast 4 days and placed in a metabolism cage. 3 days

later 8 cc. of sterile (autoclaved) dog bile were injected into the pancreatic duct under morphine and ether anesthesia. The animal remained clinically well under observation during the following 9 days, and the abdominal wound healed promptly. 6 weeks later the pancreas was examined and showed slight scarring and puckering.

*Dog 16–175* (*Table VI*).—Young dog, male; weight 23.5 pounds. The dog was allowed to fast 5 days and placed in a metabolism cage. 2 days later, under morphine and ether anesthesia 10 cc. of sterile (autoclaved) dog bile were injected

TABLE IV.

*Dog 16–46. Acute Pancreatitis (Sterile Bile in Pancreatic Duct).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | °C. | *lbs.* | |
| 1 | 3.56 | 110 | 38.0 | 25.2 | Fasting 5 days. |
| 2 | 3.47 | 80 | 38.2 | 24.8 | |
| 3 | 3.30 | 75 | 37.9 | 24.3 | |
| 3 | Sterile bile injected into pancreatic duct. Duct tied. | | | | |
| 4 | 3.86 | 132 | 39.2 | 23.6 | Slight intoxication. Feces and vomitus nitrogen 2.13 gm. |
| 5 | 5.31 | 126 | 38.7 | 23.1 | Clinically normal. |
| 6 | 4.68 | 107 | — | 22.9 | Wound clean. |
| 7 | 4.19 | 112 | 38.1 | 22.6 | |
| 8 | 4.07 | 96 | 38.0 | 21.9 | |
| 9 | 3.95 | 85 | 38.1 | 21.6 | Wound firm. |
| 10 | 4.34 | 66 | 38.2 | 21.4 | |
| 11 | 4.03 | 124 | 38.1 | 21.1 | |
| 12 | 3.78 | 80 | 39.7 | 20.7 | |
| 13 | 3.50 | 86 | — | 20.3 | Feces discarded. |
| 14 | 3.84 | 85 | 38.2 | 20.0 | |

into the pancreatic duct. The animal showed no clinical signs of intoxication and the experiment was discontinued on the 8th day. The skin incision developed two small stitch abscesses with about 1 cc. of pus but did not break down and healed promptly. 1 month later the pancreas was examined and showed a few indefinite scars. There were old calcified fat necroses in the gastrohepatic omentum.

The preceding three experiments (Tables IV, V, and VI) show the remarkable capacity of the normal pancreas to repair a serious injury. There can be no doubt about the actual injury done the gland by in-

TABLE V.

*Dog 16–172. Acute Pancreatitis (Sterile Bile in Pancreatic Duct).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | °C. | *lbs.* | |
| 1 | 1.48 | 130 | 38.0 | 13.4 | Fasting previous 4 days. |
| 2 | 1.59 | 58 | 37.9 | 13.1 | |
| 3 | 1.61 | 90 | 37.8 | 12.4 | |
| 3 | Sterile bile injected into pancreatic duct. | | | | |
| 4 | 1.85 | 92 | 38.7 | 12.5 | Not sick. |
| 5 | 2.04 | 65 | 38.9 | 12.4 | |
| 6 | 2.04 | 86 | — | 12.1 | |
| 7 | 1.88 | 63 | 38.0 | 12.1 | Wound firm. |
| 8 | 2.02 | 55 | 37.9 | 11.8 | |
| 9 | 1.99 | 59 | 38.0 | 11.6 | |
| 10 | 1.72 | 40 | 38.1 | 11.4 | |
| 11 | 1.82 | 54 | 38.3 | 11.3 | |
| 12 | 1.96 | 36 | — | 11.0 | Discontinued. |

TABLE VI.

*Dog 16–175. Acute Pancreatitis (Sterile Bile in Pancreatic Duct).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm* | *cc.* | °C. | *lbs.* | |
| 1 | 2.76 | 72 | 38.4 | 21.5 | Fasting 5 days. |
| 2 | 2.39 | 58 | 38.3 | 21.1 | |
| 2 | Sterile bile injected into pancreatic duct. | | | | |
| 3 | 3.11 | 452 | 38.8 | 20.9 | Clinically well. |
| 4 | 2.98 | 132 | — | 20.8 | |
| 5 | 2.44 | 100 | 38.3 | 20.4 | |
| 6 | 2.18 | 64 | — | 20.0 | |
| 7 | 2.23 | 56 | 38.2 | 19.8 | |
| 8 | 2.23 | 56 | 38.1 | — | Wound shows two stitch abscesses. |

jecting sterile bile into the pancreatic duct as the gland was in all cases observed for 5 to 10 minutes after the injection when the edema and hemorrhage were very conspicuous. The clinical reaction in these cases is slight and the increase in nitrogen elimination in the urine is inconspicuous in two cases (Tables V and VI) but very distinct in one experiment (Table IV). In the last experiment the pancreatic duct was tied close to the duodenum after the injection of bile had been completed, and this undoubtedly delayed the drainage of the ducts and intensified the reaction. The experiment illustrates the well known capacity of the pancreatic and bile ducts to establish continuity after simple ligature and crushing.

*Dog 16–165* (*Table VII*).—Large black mongrel sheep dog, male; weight 43 pounds. The dog was allowed to fast 4 days and placed in a metabolism cage. 2 days later, under morphine and ether anesthesia 10 cc. of dog bile were injected into the pancreatic duct. Animal showed clinical evidence of intoxication on 2nd day following, with vomiting and slight diarrhea which lasted a week. The abdominal incision was obviously infected from the 3rd day following operation and gradually broke down with necrosis of the deeper tissues. The animal became progressively weaker and was killed.

*Autopsy.*—There was a necrotic sloughing abdominal wound closed off at the bottom by omentum. Peritoneum clean except for adhesions about pancreas. Lymph glands near pancreas enlarged. Pancreas much indurated and scarred with numerous fat necroses in surrounding tissue varying up to 2 mm. in diameter. These were translucent and obviously organizing at the margins. On section the parenchyma was opaque and gray. No hemorrhages or evidence of an acute process noted. Microscopic sections show areas of cell increase and organization scattered throughout the parenchyma of the gland. Mononuclear cells predominate, but many polymorphonuclears are present. There is considerable increase in connective tissue with distortion of gland architecture. Kidney in gross and microscopically shows only cloudy swelling.

*Dog 16–173* (*Table VIII*).—Old active dog, adult male; weight 26.5 pounds. The dog was allowed to fast 5 days and placed in a metabolism cage. 2 days later, under morphine and ether anesthesia, 8 cc. of dog bile were injected into the pancreatic duct. The bile was not sterile. 3 days later the dog was dull, vomited slightly, and appeared somewhat toxic. The next day the wound was swollen and tender and on the following morning was opened, allowing bloody creamy pus to escape. There were necrosis and moderate tissue destruction. During the following week the animal became weaker and more lethargic, developed a slight diarrhea, and on the 14th day after the operation had a convulsion and was killed.

*Autopsy.*—The pericardium shows numerous small subpericardial ecchymoses and a small early patch of organizing pericarditis over the left ventricle. Beneath the endocardium of the left ventricle, in the mitral valve, and through the myocardium are hemorrhages. Sections show scattered focal necroses with polymorphonuclear infiltration around them and an increase of leukocytes in the capil-

TABLE VII.

*Dog 16–165. Acute Pancreatitis (Infected Bile in Pancreatic Duct) with Wound Infection.*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | °C. | *lbs.* | |
| 1 | 3.64 | 100 | — | 39.8 | Fasting previous 4 days. |
| 2 | 3.32 | 90 | — | 39.0 | |
| 2 | 10 cc. of bile injected into pancreatic duct. | | | | |
| 3 | 3.70* | 500* | 38.7 | 37.9 | Feces nitrogen 0.87 gm. |
| 4 | 6.00 | 710 | 38.9 | 37.0 | Slightly toxic. |
| 5 | 11.30† | 565 | 38.6 | 36.8 | Wound edematous and swollen. |
| 6 | 11.09† | 435 | 38.9 | 35.5 | " " " " |
| 7 | 11.84† | 530 | 38.5 | 33.6 | |
| 8 | 9.02† | 570 | 38.5 | 32.8 | Wound infected and discharging pus. |
| 9 | 8.22† | 600 | 38.2 | 32.0 | |
| 10 | 9.49† | 510 | 38.2 | 30.8 | |
| 11 | 4.99* | 300* | — | 29.9 | |
| 12 | 11.79 | 780 | 38.2 | 29.3 | |
| 13 | 8.96 | 540 | 38.1 | 28.4 | |
| 14 | 9.24 | 660 | 38.0 | 27.4 | |
| 15 | 6.72 | 510 | — | 26.9 | |
| 16 | 6.76 | 490 | — | 26.1 | |
| 17 | 6.33 | 555 | 38.0 | 25.6 | Blood non-coagulable nitrogen 247 mg., urea nitrogen 173 mg. |

* Part lost.

† Contains vomitus and fluid feces.

laries beside the small myocardial hemorrhages. The lower surface of the diaphragm is the seat of an organizing peritonitis and the liver is adherent to the laparotomy wound. Pancreas is semitranslucent and firm. Lobulation is obscured and chalky fat necroses are numerous. The lymph glands adjacent are all enlarged. Microscopic sections show diffuse periacinal increase in stroma

which contain foci of wandering cells. Fat necroses show some marginal organization.

The preceding experiments (Tables VII and VIII) show the results of injecting infected bile into the pancreatic duct. Under these

TABLE VIII.

*Dog 16–173. Acute Hemorrhagic Pancreatitis (Infected Bile in Pancreatic Duct) Complicated by Wound Infection, Peritonitis, Pericarditis, and Myocarditis.*

| Day. | Urine. | | Feces nitrogen. | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | | |
| | *gm.* | *cc.* | *gm.* | °C. | *lbs.* | |
| 1 | 3.11 | 85 | 0 | 38.5 | 23.8 | Fasting 5 days. |
| 2 | 2.83 | 70 | 0 | 38.4 | 23.4 | |
| 2 | Bile injected into pancreatic duct. | | | | | |
| 3 | 4.93* | 935 | 1.08 | 39.2 | 22.3 | |
| 4 | 6.17 | 818 | 0.49 | 39.3 | 21.7 | |
| 5 | 7.48 | 902 | 0 | 38.9 | 21.6 | |
| 6 | 7.06 | 870 | 0 | 39.0 | 21.0 | Wound tender and swollen. |
| 7 | 7.31 | 577 | 0.92 | 39.3 | 20.7 | |
| 7 | Badly infected laparotomy wound opened. | | | | | |
| 8 | 7.46 | 605 | 0.59 | 38.9 | 20.0 | |
| 9 | 6.97 | 605 | ± | 39.0 | 19.3 | |
| 10 | 6.36 | 577 | ± | 38.5 | 19.0 | |
| 11 | 5.12 | 575 | 0.50 | 38.4 | 18.8 | |
| 12 | 4.00 | 505 | ± | 38.5 | 18.3 | |
| 13 | 3.75 | 480 | ± | — | 18.1 | |
| 14 | 3.70 | 460 | 1.34 | 38.7 | 17.6 | |
| 15 | 3.56 | 356 | ± | — | 17.0 | |
| 16 | 3.78 | 360 | ± | — | — | Killed. Blood nitrogen 66.1 mg., urea nitrogen 26.4 mg. |

* Vomitus included.

conditions the urinary nitrogen shows a rise of 100 to 300 per cent above normal. From the data at hand we can assume that a considerable part of this rise in urinary nitrogen is due to the suppurative processes in the abdominal wall. Pancreatic injury is more pro-

nounced than in the experiments with sterile bile where the repair was more rapid and perfect. The non-protein nitrogen of the blood is greatly increased in amount and speaks for the great amount of tissue autolysis which must have been present.

*Dog 16–109* (*Table IX*).—Strong fox-terrier, adult male; weight 20.5 pounds. The dog was allowed to fast for 6 days. After a short preliminary period the dog

TABLE IX.

*Dog 16–109. Acute Pleuritis (Turpentine).*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | °*C.* | *lbs.* | |
| 1 | 2.21 | 90 | — | 18.7 | Fasting 6 days. |
| 2 | 2.35 | 105 | — | 18.4 | |
| 2 | Turpentine, 1.25 cc., injected into right pleura. Morphine $\frac{1}{8}$ grain. | | | | |
| 3 | 2.27* | 145 | 38.6 | 18.5 | Vomited. |
| 4 | 4.31 | 335 | 39.1 | 17.7 | |
| 5 | 5.80 | 250 | 39.3 | 17.2 | Clinically sick. |
| 6 | 4.73 | 210 | 39.2 | 16.9 | |
| 7 | 4.68 | 164 | 39.3 | 16.6 | 5 p.m. 50 gm. of cane sugar by stomach tube. |
| 8 | 4.70 | 538 | 38.6 | 16.1 | Vomited. |
| 9 | 4.20 | 110 | 38.2 | 15.7 | Feces nitrogen 0.56 gm. |
| 10 | 3.81* | 114 | 38.0 | 15.2 | Diarrhea. |
| 11 | 3.47* | — | 37.6 | 14.9 | " |
| 12 | — | — | — | — | Died. *Autopsy.*—Hemorrhagic pleurisy, pneumonia, tissue necrosis. |

* Feces and cage washings included.

was given morphine, and turpentine, 1.25 cc., was injected into the right pleural cavity. The dog was clinically sick during the following week, vomiting at times. During the last 2 days of life there was considerable diarrhea. Dog died during the night and was autopsied the following morning.

*Autopsy.*—The right pleural cavity contains 250 cc. of bloody fluid and blood clots. The pleural surfaces are smooth. The lungs collapsed. The upper two lobes are firm, dark red, and quite airless. One portion of this lung tissue shows complete necrosis. Microscopic section shows necrosis and hemorrhage. The necrotic areas contain great numbers of polymorphonuclear leukocytes and very

little fibrin. There is no solution of tissue. The rest of the autopsy is not important to this experiment.

*Dog 17–22* (*Table X*).—Small mongrel, male. The dog was allowed to fast for 4 days. After a short preliminary observation the dog was given morphine and a thick emulsion of aleuronat, 10 cc., was given into the right pleural cavity. This emulsion contained large numbers of *B. coli* and a recently isolated streptococcus. Following this injection the dog was clinically sick with occasional vomiting. The dog was killed.

*Autopsy.*—Performed at once. The right pleural cavity is dry and sticky. It contains little fibrin, and shows considerable injection of its surface. It con-

TABLE X.

*Dog 17–22. Acute Pleuritis and Pneumonia.*

| Day. | Urine. | | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | Nitrogen. | Amount. | | | |
| | *gm.* | *cc.* | °C. | *lbs.* | |
| 1 | 3.35 | 130 | 39.2 | 13.3 | Fasting 4 days. |
| 2 | — | — | 38.4 | 13.1 | |
| 3 | 2.86 | 130 | 38.2 | 12.9 | |
| 3 | Streptococcus and *B. coli* with aleuronat injected into right pleura. Morphine $\frac{1}{8}$ grain. | | | | |
| 4 | 4.31 | 115 | 39.8 | 12.7 | Cough. Vomited. Sick and dull. Feces nitrogen 0.84 gm. |
| 5 | 4.58 | 317 | 39.1 | 12.4 | Sick and dull. |
| 6 | 4.86 | 400 | 39.5 | 12.0 | " " " |
| 7 | 5.68 | 225 | 39.8 | 11.5 | " " " Killed. *Autopsy.*—Acute dry pleurisy and pneumonia. |

tains about 2 cc. of thick, slimy, pinkish exudate. The right lower lobe of the lung is solid, pale, and gelatinous. It is quite airless and moist on section. There are no abscess cavities. There is a uniform pneumonia involving the entire lobe. The upper lobes show a few similar areas of consolidation. The left lung is negative. The rest of the autopsy is unimportant for this experiment.

*Dog 17–11* (*Table XI*).—Large mongrel hound, male; weight 41.5 pounds. The dog was allowed to fast for 6 days. After a short preliminary observation the dog was given morphine and a thick aleuronat suspension inoculated with *Staphylococcus aureus*. This was injected into the right pleural cavity. After a slight clinical reaction the dog appeared perfectly normal. After a period of 8 days a suspension of aleuronat with a heavy emulsion of staphylococcus was injected

into the left pleural cavity. This injection likewise caused little inconvenience although a considerable rise in nitrogen elimination. After a period of 5 days the dog was given morphine and a mixture of aleuronat paste with 0.75 cc. of tur-

TABLE XI.

*Dog 17–11. Acute Pleuritis (Staphylococcus) and Peritonitis (Turpentine).*

| Day. | Urine. Nitrogen. | Urine. Amount. | Temperature. | Weight. | Remarks. |
|---|---|---|---|---|---|
| | *gm.* | *cc.* | *°C.* | *lbs.* | |
| 1 | 3.65 | 100 | — | 37.4 | Fasting 6 days. |
| 2 | 4.03 | 96 | 38.1 | 36.8 | |
| 2 | *Staphylococcus aureus* and aleuronat injected into right pleura. Morphine $\frac{1}{6}$ grain. | | | | |
| 3 | 4.94 | 105 | 38.9 | 36.1 | Very slight intoxication. |
| 4 | 5.19 | 125 | 38.6 | 35.7 | Apparently normal. |
| 5 | 4.40 | 100 | 38.2 | 35.4 | |
| 6 | 3.96 | 112 | 38.4 | 35.0 | |
| 7 | 3.57 | 106 | 37.8 | 34.2 | |
| 8 | 4.18 | 103 | 37.4 | 33.9 | |
| 9 | 4.17 | 88 | 37.5 | 33.7 | |
| 10 | 4.33 | 116 | 37.5 | 33.4 | |
| 10 | *Staphylococcus aureus* and aleuronat injected into left pleura. Morphine $\frac{1}{6}$ grain. | | | | |
| 11 | 5.68 | 170 | 38.9 | 33.0 | Apparently normal. Feces nitrogen 0.47 gm. |
| 12 | 5.46 | 170 | 37.9 | 32.3 | |
| 13 | 4.23 | 145 | 37.9 | 31.9 | Not clinically sick. |
| 14 | 4.33 | 175 | 37.9 | 31.2 | |
| 15 | 5.13 | 215 | 37.3 | 30.7 | |
| 15 | Turpentine, 0.75 cc., and aleuronat injected into peritoneum. Morphine $\frac{1}{6}$ grain. | | | | |
| 16 | 5.99 | 245 | 38.5 | 31.2 | Feces nitrogen 0.48 gm. |
| 17 | 8.82 | 345 | 38.2 | 30.5 | Abdomen rigid. |
| 18 | 7.06 | 272 | 38.3 | 29.9 | Not sick. |
| 19 | 6.41 | 243 | — | 29.2 | Killed. *Autopsy.*—Acute pleuritis, endocarditis, and peritonitis. |

pentine injected into the peritoneal cavity. The dog did not appear clinically sick, although the abdomen was somewhat rigid. The dog was killed in spite of the absence of clinical symptoms.

*Autopsy.*—Performed at once. The right pleural cavity is clear. There is a slight organizing pleurisy close to the hilum of the right lung. The left pleural cavity is clear. There is a slight exudate close to the hilum of the left lung. There is some atelectasis at the base of each lung. Heart shows a definite, acute, hemorrhagic endocarditis with small granular vegetation. Myocardium is clear. Peritoneum shows an organizing fibrinous exudate in the left flank. Intestines are glued loosely together. There is some injection of the serous surfaces. There is also a slight exudate close to the spleen and over the dome of the liver. There is no excess of fluid. Both kidneys show small, linear abscesses extending down through the cortex. Microscopic sections show a slight amount of pneumonia just beneath the pleura close to the hilum of the left lung.

The last three experiments (Tables IX, X, and XI) show the effect of a chemical irritant upon the serous surfaces. It is not necessary to record many experiments, as the results are so uniform. Moreover, it may be argued that an irritant like turpentine when placed in a serous cavity may injure not only the serous surfaces but adjacent tissues (lung, muscles, liver, etc.) as well. So the reaction of the abscess might be expected to recur again and again regardless of whether the injury is located in one spot in the subcutaneous tissue (abscess) or in any serous cavity (pleurisy or peritonitis). The same arguments apply even more forcibly to bacterial inflammation. We observe that the curve of urinary nitrogen is very similar if not identical in all these experiments whether the inflammation is localized (abscess, infected wound) or diffuse (pleurisy, peritonitis). The last experiment (Table XI) demonstrates that the normal pleura has the power to recover from a considerable injury and is able to dispose of a great number of pathogenic bacteria. With each injury to any serous cavity we note a rise in urinary nitrogen with a tendency to recover and return to normal if the injury is not too grave. It makes no great difference whether the injury is sterile or not, when we consider the evidence for injury of body protein.

Control of the ether anesthesia has been recorded in another paper (4) and it has been shown that 1 hour ether anesthesia will cause no recognizable rise in urinary nitrogen. Control laparotomy experiments (4) show a slight rise in urinary nitrogen when the abdominal incision heals with a minimum reaction.

## DISCUSSION.

The injury done to an animal by means of a sterile abscess may be made up mainly of two factors, (*a*) local injury of tissue by the chemical irritant, (*b*) general injury of body protein by means of toxic split products absorbed from the site of local injury. The sum of these injuries will account for most of the increase of nitrogen in the urine and the non-protein nitrogen of the blood. There is much evidence to show that by far the greater part of the excess nitrogen in the urine results from the general injury of body protein rather than from the local injury (for example, abscess pus).

A sterile abscess causes a great rise in the output of urinary nitrogen during the time of abscess formation but also during the 24 hours following the drainage of the abscess and disappearance of all clinical signs of intoxication. This recalls the familiar reaction recently described (2) following the injection of a toxic proteose. A non-lethal dose will cause an acute clinical reaction (vomiting, diarrhea, temperature fluctuation, and shock) which is over in 4 to 6 hours. The curve of urinary nitrogen excretion will show a slight rise usually during the first 24 hours after this injection but a maximum rise during the second 24 hours after the proteose injection. There is a delay in elimination of the nitrogen which presumably must result from protein injury effected by the toxic proteose. We wish to explain in the same way the delay in elimination of urinary nitrogen after the drainage of an abscess—the injury done to the body protein is not immediately followed by a rise in urinary nitrogen. We are not prepared to explain this peculiar lag in the escape of nitrogen following a toxic injury. The following paper shows that there may be a considerable piling up of nitrogenous substances in the blood during periods of acute intoxication. This suggests a rapid breakdown of protein substances but as well a slowing of the elimination by the kidney. We have no evidence of anatomical renal changes but the functional capacity of the kidney has not been sufficiently studied under similar experimental conditions.

Diuresis is noted in the experiments tabulated above and comes out very clearly when the dogs are allowed access to water at all times. Diuresis is not a noticeable factor when the dogs are on a uniform fluid intake given by stomach tube, so it may be that the diuresis noted

above is due to increased thirst or a craving of the body tissues for fluid. This may be a part of the peculiar reaction on the part of the body cells toward these various toxic split products.

Many of the experiments show clearly a summation effect following a combination of injuries or intoxications. A given injury will cause a certain increase in urinary nitrogen and when combined with some similar injury the nitrogen elimination will roughly correspond to the sum of the two separate injuries. One factor must be considered in any such grouping of injuries and that is the tolerance which may be established toward one injury by some related injury. It has been established (2) that preceding proteose injections render a dog more tolerant to subsequent injections. Also that the presence of a chronic intestinal intoxication will render a dog tolerant to subsequent proteose injections.

Peritonitis will cause a considerable rise in the output of urinary nitrogen and often a rise in the non-protein nitrogen of the blood. It is known that a general peritonitis is usually associated with a paralytic ileus, so there was some doubt whether or not the increase in protein disintegration was due to the intoxication of the ileus or of the peritonitis alone. Probably both factors are concerned but we have been able to isolate toxic proteose-like substances from the exudate of certain cases of general peritonitis. Further, the reaction of an acute pleurisy shows that a simple inflammation of a serous cavity can be associated with considerable injury of body protein and increase in nitrogen elimination.

The term "endotoxin" is still used today in many instances where it is impossible to isolate a toxin from a given bacterium. It is becoming increasingly evident that the word "endotoxin" is used to cloak the non-specific intoxication which may follow invasion by a given microorganism. The "endotoxin" in reality is a poison derived from autolysis of the host protein.

Some of the experiments given above (Tables IV, V, and VI) show the remarkable capacity of the normal pancreas to resist injury and to repair tissue destruction, as has been pointed out elsewhere (5). The surgeon often has a mistaken idea about the various inflammatory reactions of the pancreas, and assumes that drainage is necessary. Granting that drainage of this region can remove exudate, which is at least debatable, it is quite clear from experimental data given here

and elsewhere (5) that the gland can repair itself in a remarkable manner if left alone in a closed peritoneal cavity. There can be no question of the injury done to a pancreas by an injection of bile into the pancreatic duct. The reaction is rapid and at the end of 5 to 10 minutes the edema and hemorrhage are much in evidence. At the end of 24 hours the fat necroses, edema, and hemorrhages with peritoneal exudate are conspicuous, and yet these dogs will almost invariably recover unless the injury is extreme.

When a lethal amount of bile is injected into the pancreatic duct, there is a profound intoxication with death in 24 to 36 hours, too short a time to show any distinct modification of the urinary nitrogen curve. We are convinced that there is a very narrow margin between the non-fatal dose which permits recovery with but little reaction and the fatal dose which shows a profound intoxication and rapidly fatal outcome. A condition of delicate equilibrium may be assumed to exist in the normal pancreas—a small injury is promptly controlled but a large injury has a tendency to become intensified by the ferment activity of the injured gland. To state our belief in another way, we may assume that the pancreas has a large amount of ferment substance or proferments in its acini and a large amount of antiferment to maintain a normal balance. When an injury is produced which comes within the limits of control of the antiferment factor, the reaction is promptly limited and there is little intoxication. But if the injury is sufficient to overcome these limits of control, then the large amount of ferment material in the pancreas is set free to act on all proteins available and this reaction of the gland by its own cell autolysis forms sufficient toxic split products to cause fatal intoxication. Acute pancreatitis produced by a sterile irritant (bile) is a good example of an acute non-specific intoxication due to protein split products which must be derived from the proteins of the host.

When infected bile is injected into the pancreatic duct, the picture is complicated by a progressive and continuous inflammatory reaction. But in these experiments too the pancreas shows great ability to recover and return toward normal with a prompt control of the acute initial intoxication produced by the initial injury. When this initial injury is too grave, the intoxication is very acute, and if the autolytic processes get beyond body control, the dog will die in 24 to 36 hours in a characteristic condition of surgical shock.

SUMMARY.

Sterile abscess, pleuritis, and pancreatitis give a clinical reaction in the experimental animal very like the same acute inflammatory processes due to bacterial activity, provided the bacterial agents are limited to the initial location.

The curve of urinary nitrogen excretion in the fasting dog shows the same precipitous and sustained rise in sterile and bacterial inflammatory reactions. This indicates that the same type of protein injury and autolysis in the body is produced by the sterile inflammatory reaction as by the bacterial reaction.

It is assumed that the primary effect of the chemical agent or of the bacterial growth in the tissues is local cell injury or necrosis. This injured cell protoplasm undergoes prompt autolysis with escape of toxic protein split products. These toxic protein split products may be, in part at least, of the proteose group and are absorbed into the circulation, producing the familiar general reaction.

The injury of body protein is obvious from the great increase in elimination of nitrogen in the urine and appears to be the same in sterile and in bacterial inflammation. The injurious agent in the sterile inflammation must be derived from the host protein, and we may assume with safety that much of the injurious material emanating from a septic inflammation must come from the host protein rather than from the bacteria.

Acute sterile pancreatitis is one of the purest examples of an acute non-specific reaction where the intensity of the host's intoxication may reach a maximum in 12 to 24 hours. We believe that fundamentally this reaction is very similar to that observed after the production of a sterile abscess or pleurisy.

Non-specific intoxication must account for the sterile reactions described above. Septic inflammations show the same acute reaction and injury of body protein. The deduction is obvious—that a great part, at least, of the reaction in septic inflammation is truly non-specific and results from the primary injury of the host's protein and cell autolysis.

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# PROTEOSE INTOXICATIONS AND INJURY OF BODY PROTEIN.

## V. The Increase in Non-Protein Nitrogen of the Blood in Acute Inflammatory Processes and Acute Intoxications.

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In the preceding communication (1) we submitted evidence to show that suppurative processes or acute inflammation due to a chemical irritant gave the same increased output of urinary nitrogen as did the same inflammatory reaction when caused by some specific bacterial agent. This, of course, indicates a similar type of injury done to body protein with elimination of the nitrogenous end-products in the urine. It is rather striking to note the great rise in urinary nitrogen above the base-line level which may be caused by a sterile abscess or pleurisy. We can scarcely imagine that any such increase can be explained entirely by a local destruction of tissue with the elimination of the nitrogen derived from these destroyed cells, but we must assume the formation of toxic split products in the abscess area which are absorbed and cause a general intoxication, cell injury, and resultant nitrogen increase. Proteose-like substances have been isolated from these inflammatory exudates and shown to be toxic. The total amount of nitrogen in the pus of a sterile abscess will rarely exceed 1 gm. but the dog may show an excess urinary nitrogen excretion of 2 to 4 gm. per 24 hours which can scarcely be accounted for by the local injury and tissue destruction alone.

We have pointed out (2) that during the intoxication of intestinal obstruction there develops a great increase in the output of urinary nitrogen; further, (3) that with the progressive intoxication of obstruction the blood non-protein nitrogen usually shows an appreciable rise

and at times a very great rise above normal, even to 100 mg. per 100 cc. of blood or higher. This has considerable clinical significance in this condition and when the non-protein nitrogen of the blood is much above normal one can feel certain that the intoxication is serious no matter what the clinical picture may be, provided the kidneys are normal.

When it was established that inflammatory processes due to a sterile irritant were able to bring about this great increase in the output of urinary nitrogen, it seemed important to follow the blood non-protein nitrogen in certain of these experimental conditions as well as in clinical cases. The data given below show that there is a distinct increase in the blood non-protein nitrogen in a variety of acute inflammatory conditions whether due to chemical or bacterial agents. There is considerable individual variation as in the intoxication of obstruction, but as a rule there is an increase in the non-protein blood nitrogen, at times a very considerable increase above normal (Table III).

### *Methods.*

The dogs used in these experiments were kept in standard metabolism cages as described in previous articles and were fasting. The detailed protocols of most of the animals listed in Table II will be found in the preceding paper of this series. In the human blood examinations the following method was used: With a volumetric pipette 5 cc. of blood which had been drawn into a little solid sodium oxalate and shaken were added to 30 cc. of distilled water in a 50 cc. volumetric flask. To this 5 cc. of a 20 per cent solution of a freshly prepared solution of metaphosphoric acid were added, the flask was shaken, and allowed to stand at least 30 minutes. Distilled water was then added to the 50 cc. mark, the flask again thoroughly shaken, and the mixture filtered. The nitrogen in a 30 cc. sample of the filtrate, representing 3 cc. of blood, was determined by the Kjeldahl method. Duplicate estimations of blood samples were made in each instance. Urea was determined by the method of Marshall as modified by Van Slyke and Cullen (4).

## EXPERIMENTAL OBSERVATIONS.

### *Blood Non-Protein Nitrogen in Sterile Abscess Formation.*

Three dogs (Nos. 17–85, 17–86, and 17–87) were allowed to fast 5 days and each animal was given 1.5 cc. of turpentine subcutaneously over the thorax. Blood was collected daily from the jugular vein.

With the formation of the abscess typical clinical symptoms of fever and general intoxication developed with diuresis such as have been noted in the preceding paper (1). On the 3rd day after the subcutaneous injection the abscess in each animal was opened and drained. Two of the animals recovered as usual but the third (Dog 17–86) died the day following the opening of the abscess.

Table I shows in all animals a slight but definite rise in the non-protein nitrogen of the blood accompanying the formation of the abscess. Although the increase is relatively slight when compared with cases of intestinal obstruction and acute proteose intoxication,

TABLE I.

*Blood Nitrogen with Experimental Sterile Abscess.*

| Date. | Dog 17-87. | | Dog 17-85. | | Dog 17-86. | | Remarks. |
|---|---|---|---|---|---|---|---|
| | Non-protein nitrogen.* | Urea nitrogen.* | Non-protein nitrogen.* | Urea nitrogen.* | Non-protein nitrogen.* | Urea nitrogen.* | |
| *1916* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | *mg.* | |
| Dec. 7 | 36.3 | 13.4 | 33.1 | 12.2 | 36.4 | 10.8 | |
| " 9 | 38.8 | 11.3 | 37.8 | 13.8 | 33.1 | 7.6 | 1.5 cc. of turpentine subcutaneously. |
| " 10 | 37.3 | 11.0 | 44.3 | 9.0 | 50.6 | 24.6 | |
| " 11 | 48.2 | 13.4 | 41.2 | 14.0 | 46.7 | 24.6 | |
| " 12 | 42.0 | 12.6 | 51.3 | 16.5 | 53.4 | 22.4 | Abscess opened and drained. |
| " 13 | 47.2 | 10.9 | 41.1 | 13.4 | Died. | – | |

* Non-coagulable and urea nitrogen given in terms of mg. per 100 cc. of blood.

it must be remembered that the severity of the intoxication in simple abscess formation is not to be compared with the marked intoxication in intestinal obstruction (Table II).

The first group of animals in Table II illustrates again the very high non-protein nitrogen that may accompany intestinal obstruction. In the first case noted (Dog 16–138), the renal obstruction was probably the chief factor in the blood nitrogen increase, but the infection may also have augmented it. The second group furnishes examples of the increase which accompanies experimental acute inflammations of various sorts. This increase is well marked, ranging from 15 to 40 mg. per 100 cc.

TABLE II.

*Blood Nitrogen in Acute Infections and in Intestinal Obstruction.*

| Dog No. | Diagnosis. | Nitrogen per 100 cc. of blood. | |
|---|---|---|---|
| | | Non-protein nitrogen. | Urea nitrogen. |
| | | *mg.* | *mg.* |
| 16–138 | Urethral calculus; left pyonephrosis; right hydronephrosis; uremia | 190.0 | 151.0 |
| 17–29 | Isolated closed loop; intestinal obstruction; peritonitis | 228.4 | 100.5 |
| 1–53 | Intestinal obstruction | 146.2 | 74.4 |
| 17–32 | " " peritonitis | 98.0 | 60.6 |
| 17–26 | Empyema; early pericarditis | 45.9 | 16.2 |
| 16–168 | " acute endocarditis | 57.3 | 20.5 |
| 17–24 | Healed pneumonia; acute endocarditis | 50.3 | 25.5 |
| 17–14 | Experimental pneumonia; pulmonary abscess | 71.5 | 18.6 |
| 17–22 | " " | 73.6 | 14.9 |
| 16–172 | Distemper | 51.7 | 24.2 |
| 17–89 | Experimental pneumonia | 46.7 | 28.4 |

*Blood Non-Protein Nitrogen in Infections of Human Beings.*

In this series a number of cases from the medical and surgical services of the University of California Hospital was studied. Certain of the cases had chronic nephritis which was recognized clinically or at postmortem examination, while others had no acute inflammatory lesions. In order that the chronic kidney lesions might not be a confusing factor we are omitting entirely any cases in which there was a suspicion of a chronic nephritis. Autopsies were performed on all the fatal cases except two and the clinical pictures in these latter as well as in the cases which recovered were such that the diagnoses were unquestioned.

The careful clinical and pathological observations made on the cases of this series make them comparable with experimental data obtained from animals and we consider it fortunate that the series studied included cases which were so typical and relatively uncomplicated.

In a recent paper of Schwartz and McGill (5) are recorded many blood urea determinations upon clinical material. They report forty-two urea readings in twenty cases of pneumonia of which thirty-

TABLE III.

*Blood Nitrogen in Acute Infections and Intoxications of Human Beings.*

| Case No. | Date. | Clinical diagnosis. | Nitrogen per 100 cc. of blood. | |
|---|---|---|---|---|
| | | | Non-protein nitrogen. | Urea nitrogen. |
| | *1916* | | *mg.* | *mg.* |
| 1 | Sept. 27 | Postoperative pneumonia; pulmonary abscess. | 79.3 | 40.9 |
| | " 28 | | 62.3 | 37.2 |
| | " 30 | | 58.4 | 23.2 |
| | Oct. 25 | | 44.1 | 20.1 |
| 2 | Oct. 7 | Acute lobar pneumonia. | 42.0 | 20.8 |
| 3 | Nov. 11 | Acute lobar pneumonia. | 51.5 | 33.3 |
| | " 13 | | 84.9 | 49.8 |
| 4 | Nov. 10 | Postoperative peritonitis. | 58.8 | 24.3 |
| | " 13 | | 88.7 | — |
| | " 16 | | 60.2 | 24.1 |
| | " 19 | | 109.2 | 57.8 |
| 5 | Oct. 6 | Acute and chronic endocarditis; acute pleuritis; cardiac decompensation. | 38.3 | 10.9 |
| | " 13 | | 41.6 | 16.8 |
| | " 25 | | 38.8 | 10.6 |
| | *1917* | | | |
| | Jan. 31 | | 47.6 | 14.0 |
| | *1916* | | | |
| 6 | Oct. 20 | Acute and chronic endocarditis; decompensation. | 87.4 | 51.5 |
| | " 24 | | 133.4 | 63.8 |
| | " 26 | | 107.8 | 47.6 |
| | " 30 | | 107.3 | 55.4 |
| | " 31 | | 139.1 | 67.8 |
| 7 | Oct. 5 | Cancer of gall bladder; duodenal stenosis; acute bronchitis and bronchopneumonia. | 234.7 | 137.2 |
| 8 | Oct. 26 | Cancer of stomach with stenosis and tetany. | 129.7 | 66.1 |
| | " 27 | | 161.0 | 82.3 |
| 9 | Oct. 18 | Acute yellow atrophy of liver. | 56.2 | 6.7 |
| 10 | Sept. 28 | Mitral and tricuspid insufficiency; decompensation. | 35.0 | 14.8 |
| | " 29 | | 36.4 | 14.8 |
| | Oct. 6 | | 41.1 | 19.6 |
| 11 | Aug. 28 | Mitral and tricuspid insufficiency; decompensation. | 40.1 | 18.2 |
| | Oct. 6 | | 43.7 | 21.3 |
| 12 | Oct. 27 | Emphysema; tricuspid insufficiency; decompensation. | 42.9 | 17.2 |

six readings are above normal. Some readings were as high as 100 mg. per 100 cc. of blood. Three cases of septicemia showed high readings: 51, 92, and 127 mg. of urea per 100 cc. of blood.

Table III shows as a rule some increase in the blood non-protein nitrogen in pneumonia, endocarditis, and peritonitis.

Case 6 with acute endocarditis and septicemia shows a very high blood non-protein nitrogen (139.1 mg. per 100 cc. of blood) on the day of death, and the week preceding death shows a mounting curve of non-protein nitrogen. The last three cases (Nos. 10, 11, and 12) give control observations on severe cases of broken cardiac compensation and marked passive congestion of the viscera. The blood non-protein nitrogen is normal.

Cases 7 and 8 with duodenal stenosis (cancer) and pyloric stenosis (cancer) show very high blood non-protein nitrogen. The obstruction is undoubtedly in part responsible but we have some observations to suggest that the intoxication associated with cancer may at times show a rise in the blood non-protein nitrogen. It is obvious that the tissue autolysis, either cancer tissue or invaded and destroyed host tissue, may be directly responsible for this change.

Case 9 is of great interest because of the low urea figures. This person was certainly suffering from grave liver insufficiency for several days before death. The blood non-protein nitrogen is above normal, and this is to be expected with this type of liver autolysis and intoxication. Yet in spite of this increased protein catabolism which usually, as we know, breaks down to urea, in this instance the usual amount of urea could not be formed. May not this lack of urea formation be attributed to the extreme liver injury? This speaks against any great activity on the part of other body tissues in the formation of urea. We hope to supplement this observation by animal experiments which will admittedly be difficult because of the remarkable reserve capacity of the liver and its ability to regenerate after injury.

### *Protocols.*

*Case 1. Pneumonia; Pulmonary Abscess.*—F. L., woman, age 42 years, who developed bilateral pneumonia with pulmonary abscess after a pelvic operation Sept. 25, 1916. Physical signs, sputum, and x-ray examination confirmatory. Probable etiology was dislodgment of infected thrombi in pelvic veins. Larger

abscess (right) drained by thoracotomy Oct. 20. Pus showed streptococcus. Died Oct. 30. No autopsy.

*Case 2. Lobar Pneumonia.*—C. R., man, age 22 years, with typical consolidation of left lower lobe and dry pleurisy. Blood examination on 5th day of disease. Crisis on 9th day with uneventful recovery.

*Case 3. Lobar Pneumonia.*—E. B., woman, age 37 years, with typical lobar pneumonia involving left lower lobe. Severe intoxication and delirium. Died Nov. 13, 1916. No autopsy.

*Case 4. Postoperative Peritonitis.*—J. M., man, age 50 years, who had had a preparatory gastroenterostomy and a later gastrectomy for adenocarcinoma of pyloric region before blood observations were made. Following latter operation gastric fistula developed. Died Nov. 20, 1916.

*Autopsy.*—Anatomical diagnosis: Recent gastrectomy for adenocarcinoma of stomach and regional lymph nodes with breaking down of gastrectomy wound and gastric fistula; subacute peritonitis; pulmonary edema; scarring of pulmonary apices and calcification of bronchial lymph nodes on right side (obsolete tuberculosis); arteriosclerosis; chronic pleuritis; chronic perihepatitis; chronic splenitis; pulmonary emphysema.

*Case 5. Acute Endocarditis; Acute Pleuritis.*—J. C., boy, age 18 years, with chronic disease of aortic and mitral valves and badly decompensated heart with general anasarca. Had also chronic pericarditis and an acute pleuritis. Improved only slightly during 4 months' stay in hospital. Acute endocarditis not recognized clinically. Died Feb. 13, 1917.

*Autopsy.*—Anatomical diagnosis: Chronic endocarditis of mitral valve with mitral insufficiency; acute vegetative endocarditis of mitral and tricuspid valves; acute mural endocarditis of left auricle; relative tricuspid insufficiency; hypertrophy and dilatation of heart; chronic passive congestion of viscera; chronic adhesive pericarditis; acute fibrinous pleuritis; chronic adhesive pleuritis.

*Case 6. Acute Endocarditis; Streptococcus Septicemia.*—D. W., man, age 25 years, who had had arthritic attacks 5, 10, and 20 years previously, had much enlarged decompensated heart with usual symptoms and signs, slight fever, leukocytosis, and streptococcus in blood culture. Died Oct. 31, 1916.

*Autopsy.*—Anatomical diagnosis: Streptococcus septicemia; extensive acute vegetative endocarditis of mitral valve; chronic endocarditis of aortic, mitral, and tricuspid valves; aortic insufficiency, mitral stenosis and insufficiency, and relative tricuspid insufficiency; chronic fibrous myocarditis; thrombi in right auricle; hypertrophy and dilatation of heart; chronic passive congestion of viscera with general anasarca; infarction of spleen; thrombosis of veins around prostate; multiple pulmonary infarctions; acute pancreatitis with fat necroses; fatty degeneration of liver.

*Case 7. Carcinoma of Gall Bladder; Duodenal Stenosis; Acute Bronchitis and Bronchopneumonia.*—M. D., woman, age 62 years, with gastric symptoms of 3 years' duration. Examination showed dilated stomach due to pyloric obstruction. Blood examination on day of death.

*Autopsy.*—Anatomical diagnosis: Carcinoma of gall bladder with constriction of duodenum from fibrous tissue at liver hilum; metastatic carcinoma of liver; moderate dilatation of stomach; acute bronchitis and bronchopneumonia; acute cystitis; chronic pelvic peritonitis; chronic perihepatitis; arteriosclerosis.

*Case 8. Cancer of Stomach with Pyloric Stenosis and Tetany.*—J. W., man, age 60 years, with greatly dilated stomach (capacity 3,700 cc.), pyloric stenosis, and classical symptoms of tetany. Died Oct. 27, 1916, after enterostomy was performed for feeding.

*Autopsy.*—Anatomical diagnosis: Colloid carcinoma of stomach with pyloric stenosis and gastric dilatation; recent enterostomy (jejunum); gastric ulcer.

*Case 9. Acute Yellow Atrophy of Liver.*—N. B., woman, age 24 years, with jaundice of 3 weeks' duration. Admitted in comatose condition; intensely jaundiced and little history obtained. Leukocytes 74,000. Died the day blood sample was taken.

*Autopsy.*—Anatomical diagnosis: Subacute and chronic hepatitis with marked diffuse hepatic necrosis and atrophy; subacute perihepatitis; splenic tumor; acute bronchitis and bronchopneumonia; subserous hemorrhages (pleura, pericardium, peritoneum); minute focal necroses in myocardium with acute interstitial myocarditis; jaundice; marked epithelial degeneration of renal tubules; heart's blood and spleen cultures sterile.

*Case 10. Decompensated Heart.*—F. S., man, age 60 years, with chronic mitral and aortic disease with insufficiency of the valves, including tricuspid, following acute articular rheumatism many years previously; decompensation progressive for past 5 months, characteristic symptoms of cardiac failure with passive congestion and edema; no infection. Died suddenly Oct. 10, 1916.

*Autopsy.*—Anatomical diagnosis: Chronic endocarditis of mitral and aortic valves; mitral insufficiency, aortic stenosis and insufficiency, relative tricuspid insufficiency, chronic adhesive pericarditis with calcification; hypertrophy and dilatation of heart; generalized chronic passive congestion and anasarca; pulmonary edema; arteriosclerosis; chronic adhesive pleuritis; chronic perisplenitis; pulmonary emphysema.

*Case 11. Decompensated Heart.*—W. W., man, age 49 years, with chronic valvular disease, mitral stenosis and insufficiency, tricuspid insufficiency, and generalized passive congestion with edema. Tenth admission to hospital for broken compensation. No signs of intercurrent infection.

*Case 12. Decompensated Heart.*—W. H., man, age 53 years, with emphysema, chronic myocarditis, relative tricuspid insufficiency, and chronic passive congestion. Third admission for decompensated heart. No evidence of infection.

## DISCUSSION.

It is of some interest to speculate as to the accumulation of these non-protein substances in the blood in these conditions. The amount of excess nitrogen elimination in the urine does not indicate necessarily

the severity of the intoxication. Given two cases of obstruction or of proteose intoxication, we can assume with safety that the one showing the greater nitrogen elimination was more severely poisoned. But we cannot compare the intoxication of obstruction with the intoxication due to an abscess, according to the excess urinary nitrogen elimination. It is easy to compare two dogs, one with obstruction and one with a sterile abscess, both dogs showing a similar increase in urinary nitrogen, and yet the obstruction dog is much more severely intoxicated and may even be on the verge of death. We know that on the day after the relief of an obstruction or the drainage of an abscess there is a considerable rise in urinary nitrogen and a rapid return to normal clinical condition. This suggests a retention of nitrogenous products in the blood and body cells, as can be demonstrated in the blood.

Why is there this retention of protein split products in the body cells and fluids during these intoxications? There are several possibilities. One may assume that the cell protoplasm is injured and holds fast to these diffusable protein split products just as it may hold fast to fluids in very acute proteose intoxication (6). It may be claimed that the protein breakdown or autolysis is so rapid that the kidneys cannot concentrate and eliminate these nitrogenous substances as fast as they are formed. Or it may be assumed that the kidney cells are in some way injured so that the substances are not allowed to pass, for the normal kidney can take care of enormous amounts of urea injected into the blood (7). We believe it is necessary to determine whether the kidney's eliminative function for nitrogenous substances is in any way impaired in acute proteose intoxication or other intoxications and hope to report on this work in the near future. There is no anatomical evidence of any kidney injury in these conditions: at the most one can only note the appearance of cloudy swelling in the tubular portion of the kidney cortex. In most cases of experimental intestinal obstruction in dogs the kidneys will be found to be normal in gross and under the microscope.

SUMMARY.

Sterile abscess formation in the dog is accompanied by a large increase in output of urinary nitrogen and also by a small but definite increase in the blood non-protein nitrogen. All this nitrogenous material of course is derived from body protein injury and autolysis.

Septic inflammation in the dog (pleurisy, pneumonia, peritonitis, etc.) likewise shows a distinct rise in the blood non-protein nitrogen. This rise is not often so great as that frequently observed in the intoxication of intestinal obstruction.

Many acute infections in man (septicemia, peritonitis, pneumonia, etc.) show a definite rise in the non-protein nitrogen and urea nitrogen of the blood; some cases show a very great rise above normal (over 100 mg. of non-protein nitrogen per 100 cc. of blood). There may be no anatomical change in the kidney beyond the familiar picture of cloudy swelling. This does not exclude the possibility of some transient functional derangement of the kidney epithelium.

Certain obscure intoxications in man may show a considerable rise in the non-protein nitrogen of the blood, indicating a large amount of protein disintegration.

These findings must be taken into account in any clinical analysis and interpretation of high non-protein nitrogen of the blood in pathological conditions.

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